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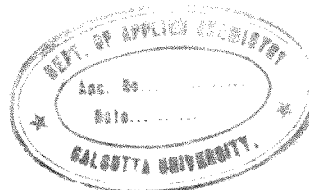
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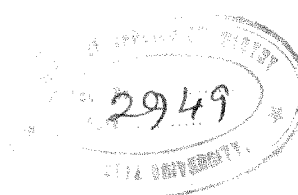
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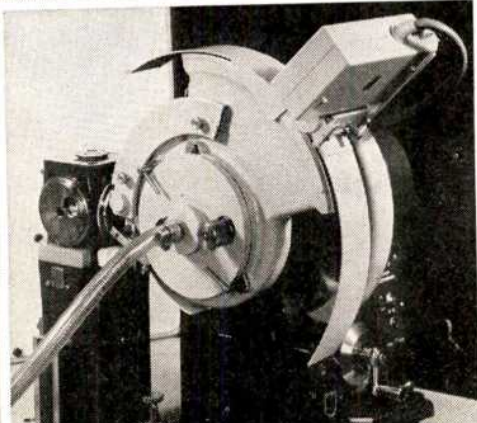
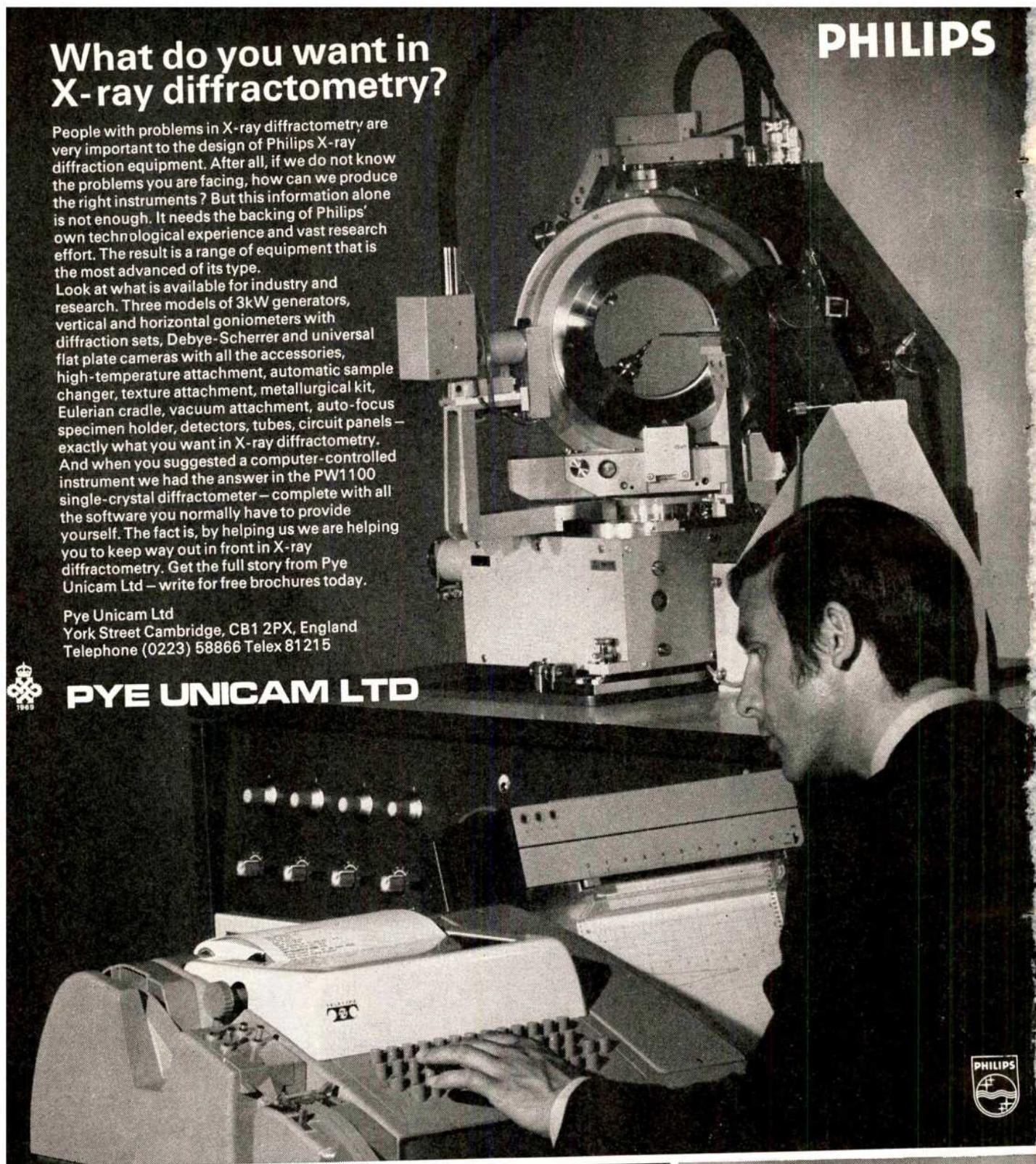
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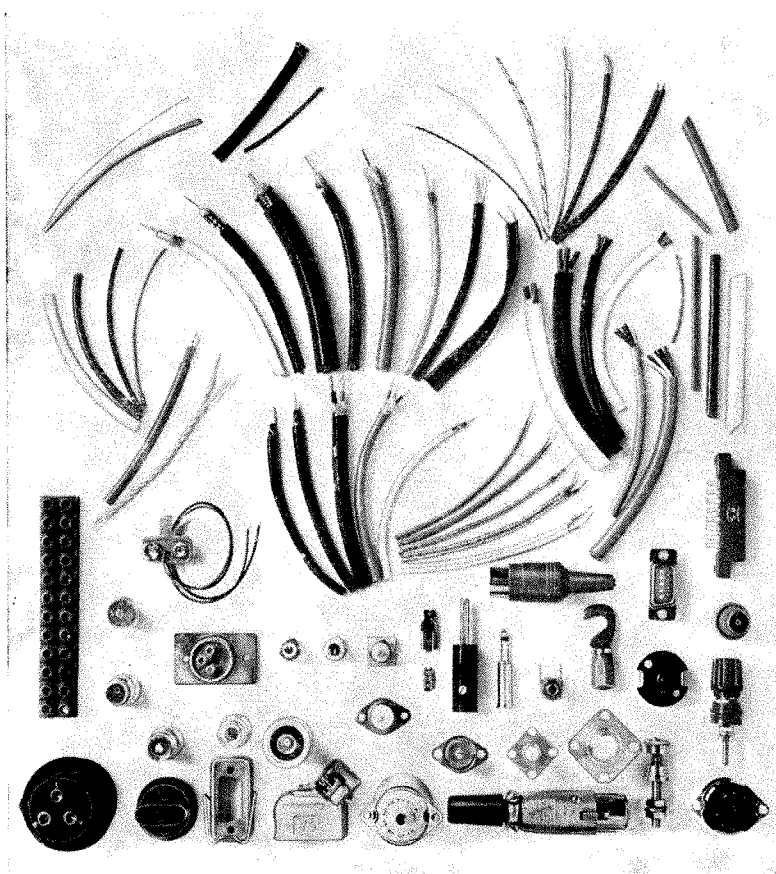
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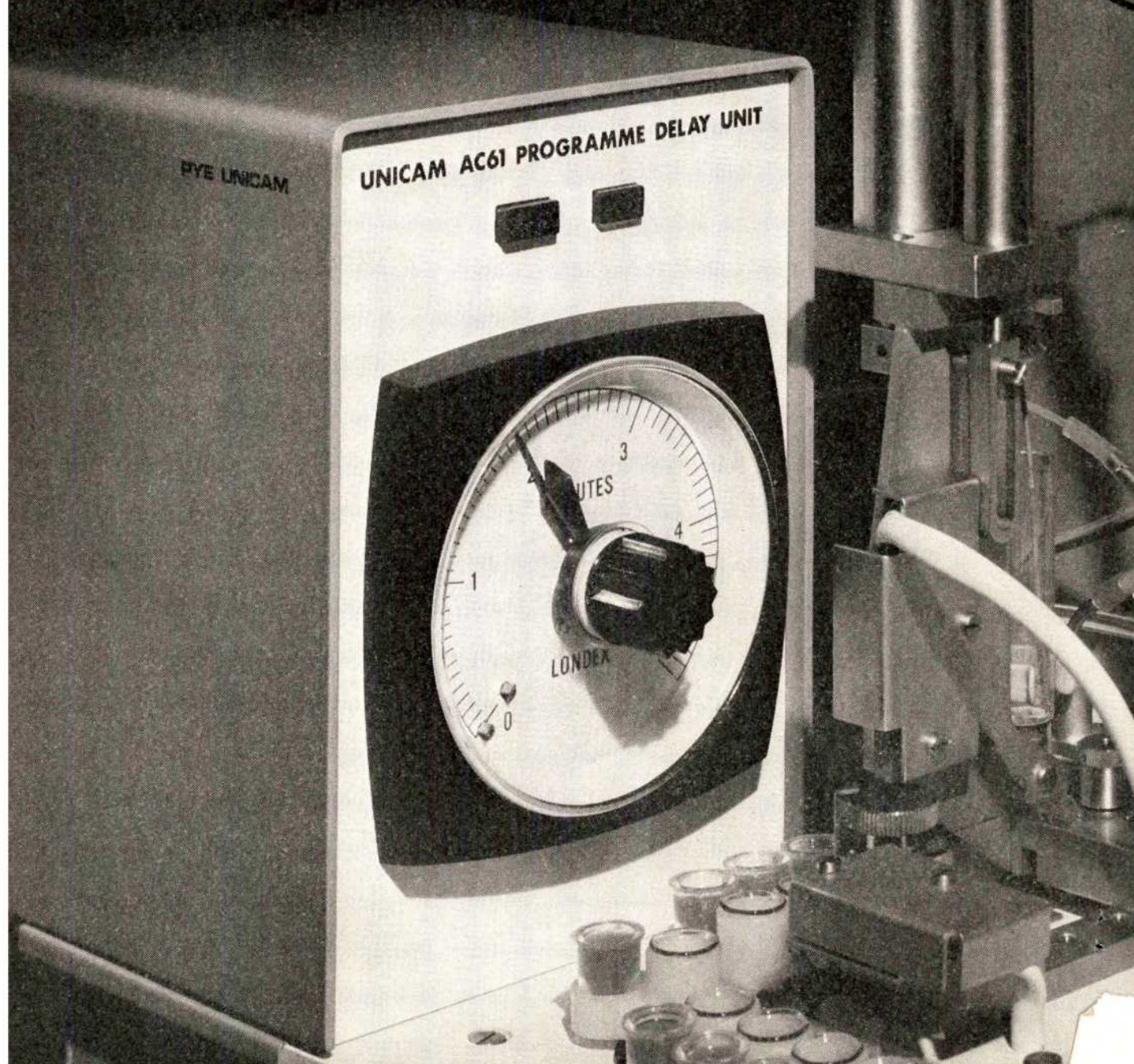
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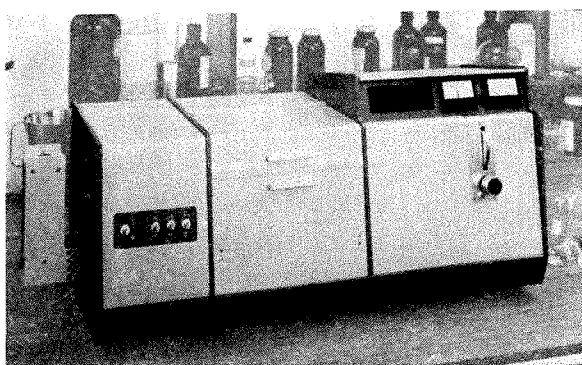


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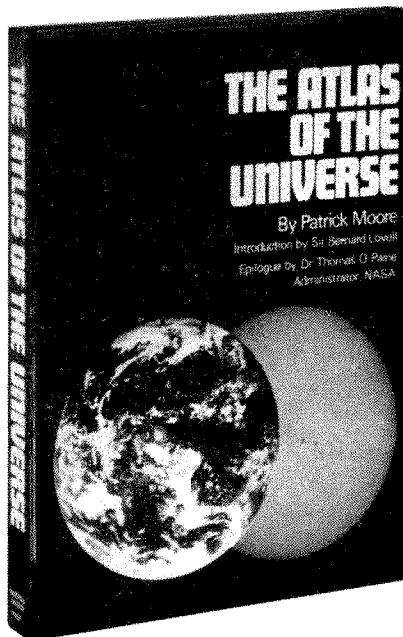
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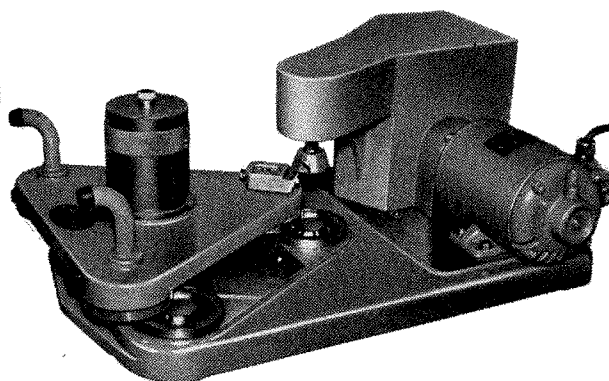


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More Watchdogs in Embryo

If the clamour for a better environment which has been dinning away in the United States for the past several months should yield no tangible result, at least there will be two new arms of the Federal Government as monuments. This, for practical purposes, is the immediate effect of the President's message to Congress on July 9 in which he asked permission—Congress has sixty days to record such dissent as there may be—to set up an Environmental Protection Agency as a separate agency of the Federal Government, and in which he also acceded to the pressures of the past year or so to set up a National Oceanographic and Atmospheric Agency—NOAA to its friends and sponsors—within the Department of Commerce. To begin with, the new agencies will be patchwork affairs, put together from the bits and pieces already in existence. In the long run, everything will depend on whether the two wholes are larger than the sums of their parts. Mr Nixon himself seems to have been well aware of the dangers of creating new departments of state whenever new problems arise, but on this occasion he has explained that the need to treat problems of the pollution of the environment as a connected whole has been overriding. There is some force in this argument, even if the physical unity of environmental pollution is not nearly as evident as Mr Nixon said. There is no necessary connexion between the monitoring and control of sulphur dioxide in the atmosphere and of sewage in the rivers, for example, but there may well be great benefits in lumping together the people who will have to be worrying about the principles on which standards for the control of both kinds of pollution are worked out.

More promising but further away, there is the possibility that those responsible for enforcing standards will gather strength by having to battle shoulder to shoulder against the vandals, for this is the sense in which the machinery of government in the United States is most grievously deficient. None of these things will happen spontaneously, however. The President's message is much that of a man who has assembled a model aircraft out of a kit of parts and who is now waiting—anxiously, it is to be hoped—to see whether it will fly. Unhappily, the United States has too long a tradition of meeting crisis by the creation of an agency for success to be assured, and there is always the danger that the concentration of responsibility for the environment in a single agency will seem to other branches of the Federal Government to be a licence to forget all about the need to look out for the environment. If the environment is indivisible, it is also by definition ubiquitous, and time may yet show that the President would have been wiser to

devise machinery for making the more feckless agencies act responsibly than to rob agencies such as the Department of the Interior and the Department of Health, Education and Welfare of those functions which already make it rub their noses in the environment, so to speak. Time will tell, but the success is neither automatic nor—on precedent—likely.

The President has not gone out of his way to make sure that the new agency will be sufficiently well endowed to carry weight in Washington—even his friends will admit that. Why, for example, should the new environmental agency be given that part of the Food and Drug Administration which is responsible for setting standards for the regulation of pesticides, but not that which controls food additives? (That remains with the FDA where it has been since the early fifties.) No doubt there are those who will argue that such a course will help to avoid fiascos such as that earlier this year in which the FDA and the Department of Agriculture took opposing views on the safety of the herbicide 2,4,5-T, but they can be assured that the next muddle will be different from that. The plain truth is that if the unity of the problem of environmental control is as real as Mr Nixon makes out, there is very little excuse for not having transferred the whole of the FDA to the new agency. And what of the Geological Survey, with its present responsibility within the Department of the Interior for offshore drilling and pipelines across Alaska as well as for a proper classification of the Ordovician and the like?

The Ash Commission on Government Reorganization which has provided the framework for Mr Nixon's proposals seems to have argued for an even larger rag-bag of functions, and there is certainly a danger that, as things are, the new agency will find that it represents too small a proportion of its potential scope to be viable in the cut-throat competition not merely for funds but for leverage which occupies much of the Washington calendar. As a piece of administration by reshuffling, therefore, the best thing to be said of the new proposal is that it removes from the Atomic Energy Commission responsibility for regulating the manner in which radioactivity is released to the environment from nuclear power stations. Although there is no reason to think that the AEC has been worse than smug in its handling of these matters, it is better that the functions of judge and jury should be separated from each other. In short, there is enough doubt about the good sense of the proposed arrangements for a congressional inquiry—even a benevolent inquiry—to be a public service. It is to be hoped that sixty days will not pass entirely in silence.

Important questions about the regulatory powers of

the new organization also cry out to be answered. In spite of all the frenzy of the great campaign against pollution, the plain truth remains that the most serious difficulty in the United States is that of arranging that the aspirations of the Federal Government will be translated into action by state and municipal authorities. It is only natural that even the most vigorous advocates of a better environment should be ill-disposed towards higher taxes, but the result is usually an unfortunate separation of the analysis of problems and responsibility for their solution. This, for example, is why it is more than likely that Mr Nixon's own trumpeted programme for the abatement of water pollution, the pride of February, is now likely to seem like an ironical demonstration that the nation that won an international reputation for its domestic hygiene in the thirties is now incapable of preventing itself from using rivers as sewers, \$4,000 million of federal money notwithstanding. There is nothing in the President's message to suggest that the Environmental Protection Agency will be better able than, say, the National Air Pollution Control Administration which, in its present incarnation, has had remarkable success in winning motor car manufacturers to its way

of thinking but which has not been able to rid the United States of noisome air except in places where people do not congregate. A part of the trouble, of course, is that the control of pollution is an even hotter potato than anxiety about the environment, and that no federal government is ever likely to delegate to a separate agency responsibilities which might cut across the existing lines of demarcation between central and regional authority.

Congress, which is likely to applaud the new proposals, would certainly turn nasty if the Administration went too far in its assumption of power to regulate. The result of this tacit agreement not to give mutual offence, however, is that the embryo tiger will have no teeth until somebody manages to rob regional public authorities of their traditional right to create a public nuisance (or several) if their taxpayers choose. That, of course, is a political problem. It is no disrespect to say that the President has ducked it this time round. None of his predecessors has had much relish for it either, yet it is a cruel irony that neglect of this central problem should not be regarded as a disqualification of declarations of good intentions on the environment.

Government by Committee

IN the memorable words of Mr Harold Wilson about the opinion polls before the last election, the new leader of the new House of Commons, Mr William Whitelaw, failed to distinguish himself in his statement on the future of the select committees on July 9. The particular blend of cockiness and silliness with which he assured his colleagues that "we must avoid developments which will lead to a position like that reached in the United States Congress, where committees are all-important" may be attributed to the unexpected novelty of his present responsibilities. The consequences could, however, be unfortunate (see page 217). The occasion for Mr Whitelaw's declaration is the need to respond to the suggestions which the Select Committee on Procedure has made about the organization of parliamentary business, and it is at least creditable that he should wish to say something. The signs are, however, that the leader of the House of Commons is not entirely clear in his own mind what the House of Commons is meant to accomplish. And for all his assurance that the hard work of the Select Committee of Science and Technology will not be wasted in any reorganization there may be, he seems sadly to have misunderstood the value of what this and other committees have been doing in the past few years. It is to be hoped that his friends will disabuse him of his error before too much damage has been done.

To begin with, it is grievously mistaken to suppose that the function of legislative bodies such as the House of Commons is largely to engage in debate. In Britain, it is indeed notoriously the case that debates on important issues are occasions on which two oppos-

ing sides can state opposing and usually familiar points of view. Rarely is one of the debates after which Mr Whitelaw hankers likely to produce a result which cannot be predicted. For all his scorn of the United States Congress, this is a matter on which the Senate at least has an obvious edge, as the passing of the Cooper-Church Amendment (right or wrong) must demonstrate. The formal proceedings of the House of Commons are most constructive when some knowledgeable member is able to suggest how the government of the day might better its intentions—even if the minister does not listen, his civil servants will. But the House of Commons is much less good than it should be at keeping up informed and possibly constructive commentary on government policy. The trouble, of course, is that informed questioning of policy in some narrow area requires a degree of application which is usually beyond the scope of the randomly assorted membership of the House of Commons. The result is that on technical matters—not necessarily in science and technology—the House of Commons is less effective than it should be at uncovering and understanding the reasons why governments embark on particular courses of action. Wise leaders of the House of Commons would recognize that, in the long run, governments as well as oppositions are the losers.

A few illustrations taken from the record of the Select Committee on Science and Technology should be enough to convince Mr Whitelaw of the truth of that. The first investigation which the committee carried out led to a much better public understanding of the difficulties to be surmounted in transferring to private

industry public control of all the details of reactor development, even though its own policy recommendation (for an even more powerful central control of development policy) was something of a joke. The fact that the previous government muffed the reorganization is also beside the point (and it is probably significant that neither the Government nor the Opposition thought fit to refer to this hanging problem in the debate on the Queen's Speech last week). The value of the investigation was to have brought into the open a great deal of information that would otherwise have remained buried. Although the process is probably educative of governments, its more immediate

value is in the analysis which it provides for outside interest in their relationships with government. The point is more better illustrated than in the investigation of carbon fibre technology, in the course of which the old select committee made several industrial companies think hard about their intentions in this potentially important field. The fact that the select committee's efforts to uncover the skeletons in the cupboard of defence research have been comparatively ineffective—this was one of the more solid reports—is best explained by the indifference of government and taxpayers alike to the important issues which are involved.

Monsters and Poltergeists

1969 was a poor year for the Loch Ness Phenomena Investigation Bureau Limited. Only fourteen "acceptable" sightings were made, according to the Bureau's annual report, three of them supported by film. This is a miserably poor tally compared with the reports of flying saucers received by Project Blue Book of the US Air Force, which handled some 10,000 sightings in less than 20 years, an average of about 15 flying saucers a day. What is more, some 5 per cent of the American population believes they have seen a flying saucer, whereas professed belief in the Loch Ness monster is probably strongest among those with stakes in the Scottish tourist trade. Nonetheless, there are signs that the managers of the monster's affairs are beginning to learn the basic rudiments of their business, in which case sightings of the monster can be expected to become a more regular feature of the summer season.

Those who peddle the monster face disaster on two sides but are safe as long as they tread a middle path. One calamity would be if a particularly rigorous search of Loch Ness should prove that no monster exists. The other would be if some natural explanation for the sightings were actually to be found, since it would certainly fail to live up to the expectation of a pleio-saurian creature such as that portrayed on the annual report of the Loch Ness Phenomena Investigation Bureau Limited. The keepers of the faith are probably guarded quite well against these heresies. Loch Ness has been searched for more than a decade without any shred of monster coming to light, but this, of course, merely enhances the mystery, and no search, however meticulous its operation and sound its sonar, can easily prove a negative. Moreover, the Loch Ness Investigation Bureau Limited has shown its excellent sense of survival by undertaking not to capture or lay hand on the monster—the most it will do is take a tissue sample. Concern for a unique specimen is, of course, admirable, but even more credulous ages than this would have looked askance at somebody who went out to prove unicorns exist but vowed not to catch one. The other danger is that the monster may be found, and, if it be but a large eel or schoolboy prankster, fail to come up to expectation, but the

crisis could doubtless be met by an unflinching denial that this was the True Monster.

The True Monster, indeed, is likely to flourish in the years to come since its one vital pabulum is publicity, and its handlers are becoming increasingly adept at procuring this evanescent diet in the right quantities. No less than the Minotaur, the Loch Ness Monster thrives even on unfavourable publicity—this year's report quotes in full a recent article (*Nature*, 220, 1272; 1968) that criticized the monster's cognitive status, together, of course, with a line for line rebuttal. Indeed, the monster is now doing so well that even that sober newspaper, the *Financial Times*, greeted the Loch Ness Bureau's annual report with the headline, "Evidence of loch creature grows". The inhabitants of the Loch Morar district, for their part, have decided to exploit the monstrous atmosphere created by their neighbours at Loch Ness and earlier this month it was announced that Loch Morar, too, would be screened for a monster, already christened, with suitably Tolkienesque undertones, as Morag. The sponsors of the Loch Morar survey stated that the monster hunt would be a contribution to European Conservation Year, but it is hard to say whether the thought of conserving an animal that is almost certainly non-existent is intended as an elaborate satire on the conservation movement or a simple leg pull of the rivals at Loch Ness. It should not be long before Unidentified Swimming Objects are sighted in the Scottish lochs as regularly as the reports of UFOs in the United States that swamp any agency prepared to listen to them.

The triumphal progress of the Loch Ness and Morar monsters makes an interesting contrast with another attempt to investigate phenomena which most reasonable men would probably consider illusory. The Society for Psychical Research, founded in 1882, is now approaching a century of investigations into the occult without having yet produced any unassailable and repeatable evidence of extra-sensory perception, psychical phenomena or life beyond the grave. There is, however, a world of difference between the Society for Psychical Research and organizations like the Loch Ness Bureau of Investigation Limited.

Subject matter apart, the society practises a methodology of investigation which is not seriously different from that of the more successful natural sciences. The journal of the society, for example, contains sober accounts of experiments designed to falsify various hypotheses about psychical phenomena. Some of these accounts inspire sympathy, such as that of an attempt to detect psychical effects in protozoa (by willing them to move in particular directions) which concludes: "The writer did not embark on these experiments in an attitude of disbelief; on the contrary, he fully expected to obtain positive confirmation of the Richmond effect and was somewhat disappointed when he failed to do so" (*Journal of the Society for Psychical Research*, 45, 296; 1970). This report, however little surprise it may occasion, belongs to science no less than do statements such as: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." The language of the Loch Ness monster hunters, on the other hand, has nothing to do with science. Their experiments may be conducted with every modern piece of scientific apparatus that publicity requires, but because the methodology is deficient, the pursuit of the Loch Ness monster is no more a scientific endeavour than the hunting of the snark. That is why even poltergeists are scientifically more respectable than the Loch Ness monster.

100 Years Ago



Spontaneous Generation

DR. H. C. BASTIAX, who has recently called attention to the nature of the evidence before scientific men in favour of the theory of so-called spontaneous generation, has supplemented it by fresh experiments of his own. The dilemma in which the opponents of this doctrine are now placed is that they must either admit it, or else allow that a temperature of 150° C. maintained for four hours, and applied by means of liquid, is incapable of killing the germs of infusoria. Many, doubtless, of these opponents will courageously mount this horn of the dilemma, and make the requisite enlargement of their ideas on the subject of vital resistance to change. There are, however, other difficulties in the way. For instance, great difficulties are involved in the assumption that the atmosphere constitutes a storehouse of germs of all kinds ready to burst out into life on the occurrence of suitable conditions.

However small these germs may be, still they must weigh something. And there must be very many of them, seeing that there must be an immense number of kinds of germs, if a volume of air is to supply to any given infusion precisely the right kinds of germs suitable to the conditions provided by the infusion.

Now chemists are in possession of data showing that the possible amount of organic nitrogenous matter in common clear water and common good air is remarkably small—so small, indeed, that the question may fairly be asked—Is it large enough to admit of the requisite number of germs, the existence of which the vitalists assume in water and air?

From *Nature*, 2, 234, July 21, 1870.

OLD WORLD

INVENTIONS

New Broom for Patent Office

A THOUSAND patent applications are filed at the British Patent Office in an average week. Even so, the office is not yet so overloaded that it has had to follow the example of its counterparts in Holland and Germany which now defer the examination of applications as a matter of course. Nevertheless, something must be done as quickly as possible if a similar system is not to be introduced here. Patent applications are not published until they are accepted, and the delay can be up to four years from the date of filing. Even then the Patent Office is only able to carry out a comparatively limited search and examination so that the likelihood of the patent being invalid is higher than it ought to be. If legal proceedings are involved they can be protracted, causing more delay and uncertainty. This is the scene facing a committee that for the past three years has been looking at the patent system and which reported this week (*The British Patent System*, HMSO, £1 15s). Patents will become harder to get if the recommendations of the report are implemented.

The situation is becoming so drastic that patent offices are being driven to cooperate with each other. Last month Britain and nineteen other countries signed the Patent Cooperation Treaty in Washington, which establishes at least five centres to which the national offices can send patent applications for a report on novelty. This still leaves the granting of a patent to the national office, but a more revolutionary scheme under discussion is for a European Patent Office granting patents that would be valid in eighteen European countries (see *Nature*, 226, 888; 1970).

It will be several years before international cooperation becomes effective, and in the meantime the service given by the British Patent Office is becoming poorer. In the opinion of the committee, too many unnecessary patents are being granted, for example, because the patent office works on the principle of giving an application the benefit of the doubt if there is any chance of it coming under the Patent Acts. In future the committee would like to see the office have stronger powers to reject applications. To make patents more valuable the search and examination procedures ought to be tightened up, and the fees ought to be higher both to help cover costs and to discourage applicants who lose interest in their applications. Because of the delay in granting patents the committee would like to see the specification published 18 months after the claimed priority date, together with the search report. The applicant would then have to pay if he wanted to continue.

The committee is unusual in having an industrialist as chairman, Mr M. A. L. Banks, now chairman of Cammell Laird, rather than a member of the legal profession. Mr Banks sees the present state of affairs of the patent system as the steady deterioration of a valuable incentive scheme for industry. Mr Banks is also concerned about the conditions under which the patent officers work. The patent office in Chancery Lane has to be seen to be believed, he says, and examining staff are now accommodated in five different buildings. A new building for the patent office, which must be near the

National Reference Library of Science and Invention, is a matter of urgency.

Mr Banks is not unduly worried that the recommendations of his committee will make life harder for the rare private inventor. It seems that the cheap fees that they enjoy—British patent office fees are relatively low—will have to be sacrificed if the system is to be at all efficient. But the committee calls for the Department of Employment and Productivity to encourage the voluntary schemes by which firms reward employee inventors.

PARLIAMENT

Axe over Science Committee

THE wings of some of the select committees set up by the Labour Government are likely to be clipped and some committees, including the Select Committee on Science and Technology, may disappear altogether. These will be casualties of what the Leader of the House of Commons, Mr William Whitelaw, described last week as his desire to see the floor of the House of Commons as the centre of Parliament—the place where “true debate must take place”—and their demise will be part of an attempt to prevent the British system of government going the same way as the United States Congress in moving towards what is effectively government by committee.

Mr Whitelaw announced during his winding up of the debate on the Queen's speech that he intends to publish a Green Paper in the autumn setting out proposals for the future of the select committee system. He is expected to take up the suggestion of the Select Committee on Procedure that a select committee on public expenditure and administration should take over some of the functions of the select committees on nationalized industries, science and technology and estimates, and these committees will not be reconstituted. What will happen to the Select Committee on Education and Science which, like the Select Committee on Science and Technology, was caught by the election in the middle of an enquiry, is less certain.

A broad hint that the Select Committee on Science and Technology is likely to be dropped is Mr Whitelaw's assurance that all the hard and valuable work done by the select committees last session will be made available to whatever committee system the House of Commons eventually adopts. If this means that the evidence taken by the subcommittee enquiring into the computer industry will be available to some new select committee next year, then it is unlikely that the enquiry will ever end up as a report to Parliament because the evidence is likely to be out of date by the time any new enquiry gets under way. The evidence taken by the Select Committee on Education and Science is, however, more readily usable because its enquiry into teacher training will be carried on by the Royal Commission on Teacher Education which was announced in the Queen's speech.

It is ironic that Mr Whitelaw's avowed intention to transfer debate from committee rooms to the floor of the House will in fact mean that science and technology will receive even less attention in Westminster than they do at present. The removal of many scientists and technologists from the corridors of power by the election has left the House of Commons desper-

ately short of people interested in scientific affairs, and the Select Committee on Science and Technology at least brought Parliament's attention to some problems concerned with the development of technology. The committee, for example, has managed to focus considerable attention, both inside the House of Commons and elsewhere, on the structure of Britain's nuclear industry and on the potential for development of carbon fibres. Its enquiry into the structure of the computer industry had already aroused considerable interest among computer manufacturers and others. Although a decision to scrap the select committee will be in line with Conservative policy to “liberate” industry from government interference, it will be a pity if that decision also reduces the level of scientific debate in the House of Commons.

COMPETITIONS

Maths Marathon

ABOUT 100 young mathematicians have been competing in Budapest this week for honours in the Twelfth International Mathematical Olympiad. After surviving some very tricky courses in their home countries, they have been battling it out over two four-hour written marathons in which they have been awarded extra points for style and for taking shorter routes than their opponents. The contestants have been going for national as well as personal honours.

The national teams, each consisting of eight mathematicians aged under nineteen and a half, were all invited to take part by the Hungarian Government. It is the prerogative of the host country to choose which countries to invite and until four years ago, when the Olympiad was held in Yugoslavia, no Western country was asked to take part. This year, several Western European countries have been invited.

The British team was selected by the Committee for Mathematical Awards of the Mathematical Association, from a field of between 15,000 and 18,000 entrants. All contestants took part in the heats—an examination set by the Committee for Mathematical Awards—and the seventy who fared best were invited to take part in the British Mathematical Olympiad. This is a written event over a lengthy course similar to that of the international competition. Those who completed the questions with the most style and taking as few steps as possible were given the opportunity of representing their country in the International Olympiad. A British team has competed in three previous competitions and each time it has come fourth after Hungary, East Germany and the Soviet Union.

The International Olympiad consists of two four-hour papers set jointly by the countries taking part. Contestants have to answer three questions in each paper; for example, “Prove that there exists a unique triangle whose sides are consecutive real numbers and one angle of which is twice as large as another” (1968 Olympiad). Special prizes are awarded for particularly elegant solutions to some of the problems. The British team consists of Bernard Silverman (City of London School), Jonathan Edwards (Royal GS, Newcastle), Stuart Bell (Dartford GS), Charles Batty (Rugby School), John Proctor (Queen Mary School, Walsall), John Segal (Dulwich College), Stephen Furber (Manchester GS) and David Grubb (Greenford GS).

STRUCTURAL ENGINEERING

Test for Tower Power

WITH the supply of pylons for electricity transmission becoming an increasingly cut-throat business where every pound of weight saved counts, an optimistic future for the National Tower Testing Station was predicted by its manager, Mr W. R. Box, last week. If nothing else, the station wins the prize for being the most bizarre laboratory in Britain. A stone's throw from Cheddar Gorge, the station's asset is a disused limestone quarry which happened to be just the right shape and a collection of hydraulic rams dotted around the floor and face of the quarry. To simulate the working conditions of the pylons—conductors covered with ice, and so on—the rams, by working backwards, exert known loads on ropes attached to the structure. But the science involved is more than the parallelogram of forces. From a control room perched above one face of the quarry Mr Box and his colleagues run a sophisticated system which controls the rams and measures the applied loads to one per cent.



The National Tower Testing Station. Some of the rams are enclosed in the shed on the far face of the quarry.

Established by the Central Electricity Generating Board in 1966, the station is not yet making a profit, but it is now covering operating costs and expects to be completely self-supporting by March 1973. Pylons for use overseas occupy about sixty per cent of the work of the station, divided half and half between pylons designed by British companies for export and pylons designed by overseas firms. A pylon designed in Stockholm for use in Pakistan was being erected on the test pad last week. Pylons for use in Britain are going to be in the minority until a new range of towers is instituted.

Although Mr Box and his staff would like to get their hands on all kinds of structures, it looks like the testing of electricity transmission pylons will be taking up most of their time for the next five years or so. Where the tendency has been to overdesign these towers in the past, designers are now paring down the quantity of steel to go after the lucrative contracts in the developing countries of Africa and South America. Competition is fierce and with transmission lines several hundred miles long contracts are won or lost on quite small savings on cost per tower. Towers have to be

tested by law, and designers can send their towers to the National Tower Testing Station where the various disasters which can happen such as icing or an uneven load due to a broken conductor are simulated. Once a tower has been shown to live up to its specification, the designer likes to see by how much he has overdesigned it, and about fifty per cent of the towers are eventually tested to destruction.

ENVIRONMENT

Last Year's Dirty Water

OIL spillages in Britain's coastal waters, both accidental and deliberate, were worse last year than ever before. This is the gloomy news reported by the Advisory Committee on Oil Pollution of the Sea, a body which consists of representatives of the voluntary associations and other British organizations interested in marine use and conservation (report available from 1 Dorset Buildings, Salisbury Square, London EC4). Most of the oil washed up along the coastline came from unidentified sources, and the actions of unscrupulous skippers can only have been encouraged further by the absurdly small penalties imposed after conviction for illegally discharging oil—the average fine for the year was just over £225.

A tougher line with offenders is now promised by the Oil in Navigable Waters Bill, which the new government has taken over. But there are still accidents which damage wild life, and the advisory committee has set up a research unit at the University of Newcastle upon Tyne to investigate the effects of oil on seabirds. The unit is concentrating on the rehabilitation of oiled birds, but difficulties so far have meant that killing the birds remains the recommended policy. In any event, it seems that the worst oil spillages are more likely to kill birds outright—a single episode off the Dutch coast in February, for example, caused at least 20,000 deaths, chiefly of scoters and eider ducks.

Fatalities from other causes, however, were even more serious during 1969. The Royal Society for the Protection of Birds reports that nearly half the breeding population of guillemots in the Irish Sea have not returned to their cliff ledges this year, and suggests that some 50,000 may have died. About 14,000 razorbills also seem to have been lost. Although some birds may be coming back late after wintering in the open sea, these figures may well be the aftermath of the baffling appearance last autumn of thousands of dead seabirds on the shores of the Irish Sea (see *Nature*, 224, 402; 1969). Since in general only about a quarter to a fifth of birds that die at sea are washed up on the land, the 15,000 known deaths correspond fairly closely to the depletions in the breeding population. What killed the birds will probably remain a mystery—suggested explanations such as disease or unusually prolonged gales or contamination with polychlorinated biphenyls all seem insufficient to account for its scale—but the final word must await the publication in a few weeks' time of a report by the Natural Environment Research Council, which coordinated the subsequent investigations.

Polluted water inland is put into less foreboding perspective by the report of the Water Pollution Research Laboratory for 1969 (HMSO, London;

17s 6d), in which work to improve the quality of effluents complements studies of the effects of pollutants. One of the biggest projects is a study of the notorious River Trent, with the aim of finding ways to get the water cleaner without driving to ruin the industries which at present tend to treat the river as an open sewer. The chief need here is to provide by the end of the century for the extraction of 500 million gallons a day of drinkable water at Nottingham. The laboratory is also looking at the recovery of pure water from effluents; one of the most promising techniques under development involves a reverse osmosis process, which should be incorporated in a pilot plant next year.

PRODUCTIVITY

Is Your Lab Well Cited?

CALCULATING the efficiency of a laboratory is a difficult business, especially if the research is too fundamental for technological spin-off to be immediately important. A crude approach is to measure the rate at which the staff publish papers, but this method would not distinguish between the fluency of genius and the loud noises of empty vessels. An attempt to measure instead the quality of a laboratory's publications has now been made by J. Larabi of the French Centre National d'Etudes Spatiales. His method, described in *Le Progrès Scientifique* (No. 137, 1970), is to count up the number of citations that the publications have received in other people's papers.

The first application of the method has sorted out six French space laboratories into a pecking order of productivity, covering the period from 1961 to 1968. An obvious difficulty in assessing the later years is that not enough time has passed for many people to have published their references to the most recent papers. After much poring over the *Science Citations Index*, Dr Larabi has discovered what seems to be a law of propagation of scientific literature: that the rate at which a paper is cited reaches a maximum about two years after the paper is published, and that the distribution of citations through time follows the same pattern for most papers. With this as a guide, he was able to adjust the number of citations for each year to take account of those which may be expected to appear in the next few years.

Four of the laboratories immediately dropped out of the race, references to their output being relatively few and far between. That left the Laboratoire d'Aéronomie and the Groupe de Recherches Ionosphériques, of which the latter emerged as top dog in

every year except 1964 (see table). Dr Larabi emphasizes that his technique is only in its early stages, but he is now working on a cost-benefit analysis with citations as its basis, and expects to be able to surmount the awkward fact that the money spent on research in one year may be related to publications appearing several years later.

DIESEL ENGINES

Rolls-Royce Comes Clean

ROLLS-ROYCE has spelt out its claim to a larger share of the diesel engine market by becoming the first company to produce an engine to comply with the stiff antipollution regulations set out by the British Standards Institution nearly three years ago. Having entered diesel engine manufacture seriously in 1966, Rolls-Royce is hoping that the BSI stamp of approval for the mark 2 version of its 12 litre Eagle 200 engine will prove a timely fillip for its drive to capture important sectors of the market for large engines, particularly for trucks of 30 tons and over.

Apart from a series of exacting tests on vehicle performance, the BSI regulations include a strict limitation on the amount of carbon and solid matter permitted in exhaust fumes. Carbon concentration has been found to vary inversely as the square root of the gas flow, and the BSI standard is the first to incorporate a carbon limit in its demands. One of the casualties of the election has been the abandonment of a White Paper being prepared by the last government which sought to introduce smoke and noise tests into the compulsory examination which heavy goods vehicles have to undergo each year.

Rolls-Royce has made several changes in the design of the 200 h.p. Eagle engine to meet the requirements of the BSI standard. A new piston arrangement has been devised, incorporating three piston rings instead of four, and the whole engine is considerably lighter than the previous model. Production of the Eagle engine is already twice what it was only three years ago, and Rolls-Royce must be hoping that the new government will prove to be as devoted to antipollution legislation as was its predecessor. But it is still too early to know whether or not Mr Crosland's plans to curb pollution from diesel exhausts, as spelled out in the White Paper on the environment published just before the election, will prove to be yet another casualty of the change of government.

British diesel manufacturers selling abroad face lighter antipollution regulations than those laid down by the BSI. In the United States, diesel vehicles have to pass very stringent mechanical tests but there is as yet no ruling on carbon emission from exhausts. On the continent of Europe, there is also no test for carbon emission from diesel engines, but the EEC countries are now considering new proposals which are much on the lines of the BSI standard.

Legislation to limit the noise from heavy vehicles came into effect in Britain on April 1 this year, and brought the maximum permitted curbside level down from 92 dB to 89 dB. Further reductions were outlined in Mr Crosland's White Paper, including the long-term aim of cutting levels for lorries down to 80 dB and for cars to 75 dB. A reduction of 10 dB on this scale means a drop of 50 per cent in the noise which people

THE TOP TWO LABORATORIES: CITATIONS FOR EACH YEAR'S PUBLICATIONS FROM 1962, CORRECTED TO INCLUDE CITATIONS EXPECTED IN THE FUTURE

Year of publication	Number of citations in 16 selected journals Laboratoire d'Aéronomie	Groupe de Recherches Ionosphériques
1962	13	37
1963	25	42
1964	38	28
1965	40	61
1966	45	67
1967	32	78
1968	26	40

subjectively experience. The BSI regulations, which were devised at the instigation of the Ministry of Technology and Industry, make no mention of noise levels, but there will be renewed pressure now on all manufacturers to tailor their engines to the requirements of the BSI certificate.

SOVIET UNION

Problems of Application

from our Soviet Correspondent

IN the past few years there have been frequent announcements in the Soviet press of the opening of scientific institutes and research centres throughout the length and breadth of the Union. While some of these are academic "giants", many of them are relatively small units, with their research slanted at some specific local problem or development. The place of these smaller institutes in the overall picture of Soviet science, and the special planning problems which they pose, has recently been raised by the director of one such institute, I. Evgen'ev, of the Byelorussian Highway Research Institute (*Pravda*, July 7, 1970).

In Byelorussia, for example (the third largest of the Union Republics), more than half the scientific organizations number less than 200 workers. Mostly they are local institutes financed by the relevant ministries and government departments of industry, agriculture and civil engineering. A ministry tends to consider dependent institutes as "its own property", but the support which it gives them, says Evgen'ev, is "not bad at all".

A distinctive feature of these local branch institutes is the absence of any intermediate body between them and the industry they serve. The result of their activity is the implementation in practice of the results of research. In particular, they are very frequently the means by which the basic research carried out in the institutes of the Academies of Sciences and other top level institutes really get a start in life. In the Highway Research Institute, for example, only 60 per cent of the scientific staff are engaged on their own research, and the remainder are engaged in applying it. But the highly specialized nature of these small institutes is, it would seem, posing a number of administrative problems.

The planners, says Evgen'ev, approach all institutes in an identical manner, be they academic giants or small branch institutes. Expenditure and the wages bill are fixed according to the research plan, which usually forms a part of the plan laid down by the government of the republic. All other activity, including the introduction of new results into industry, is "uncoordinated science" and must be financed by the department concerned. Thus there are two plans of operation, the governmental and the departmental and, in the case of small institutes in particular, this can lead to considerable practical difficulties. Furthermore, considerable losses are often involved in the practice by which more than half the work carried out by such institutes is supported by a system of economic contracts. Under these contracts, the ministry's plan not only determines the customers but also guarantees the contract price from a centralized fund. Thus the R and D carried out by the institute is

no longer necessarily conducted on a profitable or self-supporting basis. A better scheme, it is suggested, would be that the central authorities should continue to support basic research, but that all R and D contracts should be settled between the institutes and the enterprises concerned. Only thus would the unique R and D services which these institutes can offer be seen in their proper economic setting.

The proposals which come just before the Party Conference at which top level planning for the next five years will be decided are, in short, a demand that the minor institutes of the Soviet Union, which so far seem to have been held back from operating at their optimum capacity or efficiency, should be better integrated into the R and D system.

UNIVERSITIES

Expansion with Equality

by our Education Correspondent

THE government was given a forceful reminder last week of the problems it must soon tackle in the higher education sector. The Association of University Teachers, which is wholeheartedly committed to university expansion but unable to offer many solutions to the evergreen problem of how to provide university places for 450,000 students by 1981, has warned the Secretary of State for Education and Science that the government cannot stand on the sidelines of higher education for very long (*Universities in the 1970's*, available from the AUT). The association has sensibly pointed out that the first thing the government should do is to take a look at the higher education sector in its entirety, and to abolish a few of the barriers that have grown up between different institutions. There should be more cooperation between the universities and other institutions, and the association goes so far as to advocate establishing formal links between universities and some colleges of education.

Such proposals have been aired before—even the Committee of Vice-Chancellors and Principals cast an eye on the colleges of education and wondered whether they should be turned into liberal arts colleges associated with individual universities (see *Nature*, 226, 790; 1970). But the AUT goes one step further in calling for an end to the binary system, which it calls "an unnatural creation born of financial illusions". Polytechnics, the association argues, should be able to award their own degrees, and the University Grants Committee should distribute finances not only to universities but also to polytechnics and colleges of education. This would take polytechnics out of the realm of local authorities and would bring all institutions of higher education together under the same roof. Such an arrangement would make for easier planning, and would help to remove some of the "second class" stigma that the AUT believes is attached to the non-university sector of higher education.

But the real problem is, of course, money. The AUT is adamant that the higher education sector can cater for twice as many students in ten years time and still maintain its standards only if sufficient money is made available. And that means that expenditure on the universities must reach 0.9 per cent of gross national product by 1980, compared with 0.65 per cent at

present. Professor W. V. Wallace, of the New University of Ulster and convener of the working party which drew up the report, also said that polytechnics and colleges of education must be given sufficient money to improve their staff/student ratio, and to provide better library and laboratory facilities. Without this, they will always be regarded as second class institutions.

Although the association has not offered the government a blueprint for higher education in the 1970s, it has at least said what it is not prepared to tolerate, and has handed out a few suggestions for saving costs. The AUT believes, for example, that there is some scope for more subject rationalization—"in certain subjects it makes sense to concentrate expensive staff, equipment and books in a few universities from the point of view both of teaching and research". But the AUT will predictably guard with some jealousy the staff/student ratio. Professor Wallace pointed out that universities cannot be expected to maintain their academic standards if the ratio is allowed to deteriorate, and he drew attention to the fact that courses in British universities are much shorter than their counterparts in many other countries.

BRUCELLOSIS

Paying for Clean Cows

MANY more dairy and beef herds in Britain should soon be certified free from brucellosis, an infectious disease which causes spontaneous abortions in cows and undulant fever in man. This, at least, is the hope of Mr James Prior, the Minister of Agriculture, who earlier this week announced a scheme which provides incentives for farmers to banish brucellosis from their herds.

The incentives take the form of additional payments to farmers whose herds are registered as free from brucellosis. They will receive an extra 1½d a gallon for their milk and a supplement of 37s 6d per animal will be added to their hill cow and beef subsidy. The payments are guaranteed for at least five years, by which time Mr Prior hopes that at least half the country's cattle will be "clean". The farmer may find that getting his herd registered is an expensive way of gaining these benefits—no compensation is offered for the infected animals which must be killed, and often new fences and equipment must be bought to keep the herd safe—but once his herd is declared uninfected insurance schemes are available which will make good any loss through reinfection.

Of Britain's 200,000 herds, 17,000 are already certified or qualifying as brucellosis free—a result of a voluntary brucellosis scheme set up in 1967. Since these are mainly large herds they amount to about one sixth of the animals in the national herd. However, it is estimated that another third are either free from or only lightly infected with brucellosis, and it is these that the new scheme hopes to attract. In the autumn of next year a compulsory eradication scheme, carried out on an area by area basis, is to start operating, and Mr Prior hopes that if owners of relatively free herds have come forward in large numbers a reservoir of clean cattle big enough to provide any replacements needed will have been built up.

Parliament in Britain

Queen's Speech

THE debate on the Queen's speech contained little indication that the new Parliament will be any more willing to debate science and technology than was the last. Although there was no mention of scientific affairs in the speech itself, the wide ranging debate that always takes place at the start of a new Parliamentary session gives ample opportunity for members to air their views on such subjects, but only Mr Airey Neave found himself disposed to take the plunge. He put in an impassioned plea for the Select Committee on Science and Technology to be allowed to carry on with its business, and he asked the government not to rush into any decisions about research establishments or to base its industrial research policies on the Green Paper published by the Labour Government. He also asked for the Atomic Energy Authority to be retained as a separate research organization.

On education, Mr Fred Willey, past chairman of the Select Committee on Education and Science, provided welcome relief from the bitter and protracted wrangles over Mrs Thatcher's decision to withdraw the circular requiring local authorities to submit plans for comprehensive secondary education. Mr Willey accused the Labour Government, of which he was a member, of making a "disastrous mistake" in creating a binary system of higher education. He hoped that Mr Van Straubenzee, the new Under Secretary of State in the Department of Education and Science, who was also a member of the select committee, will be able to exert some influence in getting some of the committee's recommendations accepted.

Post-Apollo Programme

MR TAM DALYELL raised the question, in an adjournment debate, of Britain's cooperation with the United States on a space station and space shuttle service. He wanted some indication from the government of its attitude to the offer from NASA of a European share in the post-Apollo programme, and whether the estimate of between 200 and 300 million dollars a year, to be provided on a European collective basis, is unacceptable. Mr David Price, Parliamentary Secretary to the Minister of Technology, said that the European governments concerned will be discussing NASA's invitation at the European Space Conference next week. Although no firm decisions are expected to be taken at the conference, Mr Price said that the British Government believes that there should be a multilateral European response to the policy issues involved, and the conference is expected to lay down formal machinery for handling discussions and eventually negotiations with the US authorities. (Debate, July 10.)

Rolls-Royce

THE government has received revised proposals from Rolls-Royce for a more powerful version of the RB211 engine, and it is considering a request for financial aid to launch the engine. This announcement was made by Mr Geoffrey Rippon, Minister of Technology, in reply to questions from Mr Tam Dalyell and Mr Robert Sheldon, and the minister said that he would make a full statement later. He declined to say whether such aid would be considered unnecessary interference by government in industry. (Oral answers, July 6.)

NEW WORLD

Pernicious Relevance

from our Washington Staff

Does the United States need a national science policy? That is the subject of hearings which have just started before Mr E. Q. Daddario's Congressional subcommittee on science, research and development. While still juggling with technological assessment (*Nature*, **226**, 894 and 998; 1970), Mr Daddario has skilfully thrown another ball into the air alongside. His latest enterprise has been to table a resolution before the House of Representatives which states that a national science policy is required to provide a basis for the coordination of scientific and related activities. If Congress passes the resolution, all Federal agencies will have one year in which to suggest how such a policy should be implemented.

No longer is the pursuit of knowledge for its own sake a respectable activity; rather must all projects be examined in the cold light of the immediate needs of society—basic research should be reduced from an end to a means. Although such is doubtless not the conclusion which Mr Daddario expects to emerge from the hearings, advice of this nature is rampant. And in introducing the hearings, Mr Daddario observed that, whether or not a new rationale is needed for science, this is certainly an appropriate time to question how the government should seek to support and direct scientific research.

"Throughout the world, but especially in the United States, the attitude toward science and technology that is the most vociferous is no longer one of awe or worship, but of fear and hostility. Science and technology . . . are the gods that failed." This was the view of Dr D. K. Price, dean of the Kennedy School of Government at Harvard University. He agreed with Mr Daddario's assessment that the relationship between science and government has been stable since 1945, but is now faced with radical alterations. In the present climate of opinion, said Dr Price, the ways in which science has hitherto been supported are no longer tenable.

How does government organize its support of science at present? Very largely, basic scientific research has been supported by using funds which were appropriated for rather more practical national objectives, such as military power, space exploration, or medical care. This support has come from a large number of government agencies, including the Department of Defense, the National Aeronautics and Space Administration, the Atomic Energy Commission, the National Science Foundation, and the National Institutes of Health. Each agency has supported whatever

research it saw fit to; there have been no restrictive policies detailing which agencies should support which topics of research.

But the writing is now on the wall; all the witnesses to testify before the subcommittee agreed that the present system has outlived its usefulness. Dr Price, however, observed that it did have good qualities that are likely to be lost in the transition to a new system—largely the generous provision of funds and the degree of freedom allowed to those spending them. But Dr A. H. Dupree, professor of science history at Brown University, did not even pay homage to the successes of the old system; he urged that a new science policy is badly needed and its introduction cannot be postponed. Following the modern trend, he argued that the relation of the DOD to basic research must be reconsidered; science must pay more attention to environmental problems; the space programme must find a role for itself with predominantly scientific objectives; and the social sciences must receive greater emphasis.

The first real live scientist to appear before the subcommittee was Dr L. A. DuBridge, director of the President's Office of Science and Technology. He said that developing a federal policy for science and technology is no easy task; everyone agrees that the progress of science and technology is important to the country, but no one has established principles which would determine just how the government should organize its support. Dr DuBridge distinguished between science and technology and pointed out that a single federal policy cannot cover both of them. Technology he defined as those research and developmental efforts which are aimed at specified practical goals and are supported to the degree that such goals are thought to be important to the federal government. Science, on the other hand, must be regarded as an activity which is supported not because there is a specific practical end in view, but because it is deemed desirable to expand the bounds of human knowledge.

From this foundation, he launched an attack on the pernicious doctrine of relevance—that scientific research should be orientated toward the needs of society. Because it is impossible to foresee which area of scientific research will yield what kind of knowledge or what kinds of application that knowledge will have, he said, any federal policy relating to the federal support of knowledge-directed science must be based essentially on the faith that knowledge as a whole is useful and beneficial to mankind. This encouraging rebuttal of present attitudes was in contrast to the

rather more pragmatic advice of Dr Price, who had previously argued that the effective justification for academic freedom and university independence is not that scholars should have special privileges; rather the types of control that raise standards of performance in industrial or administrative work would degrade standards in scholarship.

It was wrong, Dr DuBridge continued, to exclude agencies such as the DOD, AEC and NASA from supporting basic research, if only because it is impossible to define their future needs. He pointed out that the Mansfield amendment approved by Congress has stopped the DOD from supporting other than directly relevant research, and some of the university projects most valuable to the country (about \$8 million worth) have been cut out because the DOD was unable to prove their relevance to defence.

Man has had value for money so far as investment in basic research goes. According to Dr DuBridge, the total amount that has been invested in knowledge-directed scientific research throughout all history probably totals not more than about \$27 billion—and this is the basis for a trillion dollar economy in the USA. A policy for science as contrasted to technology, he concluded, must be one which simply recognizes the fact that the nation must have a strong scientific basis which must be maintained and allowed to grow with the GNP.

PUBLIC HEALTH

New York City's Health Corporation

from our New York Correspondent

THE largest non-federal hospital system in the United States was recently turned over to a quasi-public corporation. New York City has yielded up the 18 hospitals that have comprised the City's Department of Hospitals to the Health and Hospitals Corporation, in an attempt to cut through the bureaucratic red tape that has plagued the department in recent years and to increase the responsiveness of the hospitals to the needs of the communities they serve.

To meet the health needs of nearly half of the city's population, the corporation will have a budget of \$617 million in the coming year, of which \$175 million is guaranteed by the city, the rest coming from federal medicare and medicaid funds, from private health insurance payments and from various state and federal grants. The corporation will also oversee more than one billion dollars in construction that has already been approved or is under way.

Dr Joseph T. English, the head of the new corporation, has described it as "probably the most significant new venture in the development of quality health care in a large complex metropolitan area that we're going to see in this country in some time". In spite of this enthusiasm, there has been much controversy in the city over the transfer of power from a city agency to a public corporation. Critics of the new corporation claim that the city is simply abdicating responsibility for the health needs of the city, while proponents believe that an independent corporation is the only way in which health care can be made efficient and responsive to local needs.

Herman Badillo, the former Bronx Borough president, has agreed to serve on the corporation's board of directors but continues to be an outspoken critic.

In a recent speech, he stated that, "by removing the hospitals from direct government control, we're making them even more remote from the people. The mayor can say, 'It's not my responsibility.'". Another critic of the corporation is City Council president Sanford D. Garelik, the only member of the Board of Estimates to vote against the agreement between the city and the corporation.

"I voted a strong protest against the philosophy of government that the corporation represents," Mr Garelik has said. "All it does is take the hospital system farther from the people."

In reply to these criticisms, supporters of the corporation point out that reforms have been attempted in the past with little effect because of the structure of the city charter and because the hospital agency has had to deal with the central city agencies on all budget, personnel, and purchasing requests. "This has led to a tremendous middleman bureaucracy, making it impossible to have good, sound local decision-making," said Robert A. Derzon, the present deputy commissioner of hospitals. Mr Derzon, who is leaving at the end of July for a job in San Francisco, added that, "Under the old agency structure, local elected officials couldn't implement local policy even if they wanted to. We are more interested in decentralizing, not centralizing; we need strong local management and to get this we must be able to give each hospital autonomy to run its own affairs."

The present situation has grown out of a historical development unique in the United States. While most American cities have only one or two public hospitals, New York City since the turn of the century has placed much more emphasis on social services. The great influx of poor immigrants into the city led to a rapidly expanded public health care system. Public hospitals thrived during the 1920s and especially during the depression, when jobs were scarce and doctors were pleased to train in public hospitals. During the Second World War, the system slid downhill as many doctors went into the army. Afterwards the gap increased because few doctors had been trained. With limited staffs, the public hospitals were no longer able to attract specialists; it was no longer prestigious to be affiliated with the city's hospitals and they became dependent upon a large influx of foreign physicians. In 1959, more stringent accreditation regulations made it even more difficult for foreign doctors to practise in New York.

At the same time, the city itself was changing. The migration of the middle and upper middle class families for the suburbs meant fewer doctors and a declining tax base. One attempt at a solution was the affiliation programme instituted in 1960, when many of the public hospitals became teaching hospitals in association with the medical schools in the city, and when the public and private sectors of health care were brought closer together. But however finances were bolstered, the hospitals themselves did not change.

When the federal medicare and medicaid programmes came into effect in 1966, nobody was ready. There were now enough doctors, but few nurses or other paramedical personnel and few good facilities. In a city where now nearly half of the population depends on the city to provide some or all of its medical care and where four fifths of the aged and one third of the children rely solely on the public hospitals, widespread public criticism of the hospital agency led to a

number of official investigations. These brought to light much of the inefficiency and red tape and the general inability of the agency to respond rapidly to community needs. All of the commissions recommended an immediate reorganization of the municipal hospital system. The Health and Hospitals Corporation is the result.

The 18 municipal hospitals have 16,000 beds, an annual average of more than 4.7 million in-patient days and 2.9 million out-patient visits. The corporation will be one of the major employers in New York City and, with health care one of the fastest growing industries in the country, its role in the city should expand rapidly over the next few years.

To keep pace with this growth, the corporation is empowered to issue bonds, buy equipment and to bypass civil service regulations in hiring personnel. While capital investment will be tightly controlled by the city budget, the corporation will not be required to go through the Department of Public Works for construction and repairs as is presently the case. Nor will it have to use the central purchasing agency unless it wishes. In the past it has taken an average of 88 days to complete purchase requisitions and usually nine months for a hospital to hire new personnel. The corporation will delegate much greater authority to individual hospital administrators, allowing each to hire his own personnel and to spend a certain amount of money without consulting the corporation.

Advocates of the corporation feel that the elaborate system of checks and balances built into the corporation, combined with an influential and knowledgeable board of directors chosen by the city and serving five-year staggered terms, will insure that the corporation continues to be responsive and responsible to the city's health care needs. Mr Derzon claims that the board is anxious to be innovative in preventive medicine by establishing closer ties between the clinics and the hospitals, and that it hopes significantly to involve consumers. "Under the law, consumer advisory boards representative of the community are to be set up at every hospital." He recognizes, however, that the growth and development of hospitals will become political questions.

In spite of the controversy about the corporation, the prime consideration facing the corporation is whether or not it can provide the services the city needs now and in the future. However well it has been organized, its success or failure will depend on the calibre of the administrators who are running it. This is a familiar and daunting problem in the city. Many of the top members of the present hospital department have left or are leaving. Dr English will have a difficult time replacing them.

EARTHQUAKES

Death Toll in Peru

from our Geomagnetism Correspondent

THE recent earthquake in Peru (*Nature*, 226, 1087; 1970) will no doubt give geologists and geophysicists added incentive—and data—in their attempts to predict and modify such events. Only time will tell whether these efforts will succeed, but meanwhile other things can be done to reduce injuries and loss of life. The number of people killed and injured in an

earthquake depends on nature's variables—the magnitude and position of the earthquake—but it also depends on man's follies and limitations. In the Peru earthquake, as it turns out, about two thirds of the estimated 50,000 deaths were caused by the collapse of structures—mainly adobe dwellings constructed of sun-dried clay and straw materials.

Over the coastal area and in the Huaylas Canyon severe damage and collapse were restricted almost entirely to adobe constructions on unstable alluvial soil. In the hardest hit Huaylas Canyon, where 35,000 people died, about 90 per cent of the buildings are of this type. Concrete and steel structures, on the other hand, survived much better. In Chimbote, a city of 100,000 nearer the coast and the earthquake epicentre where the proportion of adobe dwellings is smaller than in the mountain villages, the proportion of deaths relative to the population at risk was much smaller (2,600).

At present there is certainly no non-collapsible wonder building which will completely eliminate earthquake deaths, and there may never be. Nevertheless the moral to be derived from the Peruvian disaster is quite clear. Construction engineers must find ways of strengthening the typical adobe construction; and new regulations should be made limiting new adobe dwellings to one storey and insisting upon light roofing materials. Ideally, of course, adobe dwellings should be eliminated altogether, but at present this is economically out of the question in Peru, Mrs Richard Nixon's visit notwithstanding.

TOLBUTAMIDE

Hazards of Leaks

THE recent furore over the use of tolbutamide to relieve diabetes has revived criticisms—never very far dormant—that the Food and Drug Administration does not take sufficient precautions to prevent the press from first unveiling to the public reports of the results of clinical trials of drug efficacy. Patients have been unnecessarily alarmed, it is alleged, by reading about the possible harmful side effects of drugs even before their doctors have been informed.

Tolbutamide is a sulphonurea drug which is given orally to treat patients with maturity onset diabetes. It was approved for use in 1957; as a result of a general review of drug efficacy, the FDA revised the labelling of the drug in 1968 to indicate that its principal value was for patients whose condition could not be controlled by diet alone. The use of other sulphonurea drugs was explicitly limited to such cases.

Together with other sulphonurea drugs, tolbutamide was reviewed for its efficacy and for its effect on diabetes complications in a clinical programme which started in 1960. The trials compared the effects of various regimes of treatment on patients in whom maturity onset diabetes had recently been diagnosed, but who were not insulin dependent and were expected to live for at least five years after their entry into the study. All patients were placed on a diabetic diet; one group was given a placebo as well; one a fixed amount of tolbutamide, and the remaining two were treated with insulin.

Two conclusions emerged from the report of the trials, received by the FDA this year. Tolbutamide

seemed to be no more effective than diet alone, and there was a statistically significant excess of cardiovascular mortality in the patients treated with tolbutamide compared with those in the other groups. The FDA does not regard these conclusions as final, however, because the trials tested the effect of a fixed amount of tolbutamide on only limited types of diabetic patient.

The details of the report have not yet been made public. Unfortunately, however, the report was disclosed to the press and the finding that mortality is fifty per cent higher after eight years of treatment with tolbutamide received the full glare of publicity. The FDA then promptly issued a press release which said that oral anti-diabetic drugs should be prescribed only for those patients who cannot be controlled by diet alone; the dose should be adjusted to the needs of the individual patient; and patients taking the drug should continue to do so unless advised otherwise by their doctor. This information was simultaneously telegraphed to doctors. The FDA is now writing in further detail to doctors, and the labelling of the drug will be changed.

What action does the FDA propose to take to prevent future such reports from seeing the light of day before doctors have been informed? Although there is concern that patients should have first heard of the hazards of tolbutamide through the press, and the problem of how to first inform doctors is being studied, no answers have been found yet. Dr C. C. Edwards, Commissioner of the FDA, said recently that "unfortunately, there is as yet no practical way of informing the physician in each case before the information reaches the patient via the lay press".

POWER

Rads or Brownouts

THERE is a critical shortage of electric power in the US, warned Dr L. A. DuBridge, the President's science adviser, in a recent address to the American Nuclear Society in Los Angeles. He said that there is "the possibility of a major failure which could produce enforced blackouts of disastrous proportions". Recent years have seen both extensive brownouts—when consumers are asked to use less electricity (people were asked to turn off their air conditioners on the hottest day of the summer last year in Washington DC) and the voltage may be reduced—and blackouts, the most spectacular of which plunged most of the north-east of the USA into darkness in 1965.

The nation's insatiable appetite for electric power, both at the consumer and at the industrial level, demands continual increases in the provision of electric

power. Table 1 shows that increases in electric power capacity are expected to run well ahead of increases in population during this century.

With increasing concern about the rapid depletion of fossil fuel resources, nuclear power has been proposed as the best answer (albeit impermanent in that nuclear resources are themselves finite). As Table 1 shows, nuclear power is expected to provide an increasing proportion of electric power.

But hassles about the effect of nuclear reactors on the environment are holding up the nuclear power plant building programme. Although nuclear reactors have certain obvious advantages over fossil fuel plants—they are free from discharges of noxious sulphur dioxide and nitrous oxide fumes and smoke—they suffer from compensating disadvantages. Because nuclear power plants are less thermally efficient than the best of the coal or oil fired plants, they discharge up to about one third more waste heat into the environment. This disadvantage may be overcome by developing new types of reactor which will operate with greater thermal efficiencies, but in the meantime there is some concern that the thermal impact of nuclear power plants may cause climatic changes.

A much more vaunted hazard of nuclear power is the risk of increasing the level of radioactivity in the environment. Although critics of the nuclear power programme have recently received publicity for their charges that the extra exposures to irradiation which could be permitted by the regulations imposed by the Atomic Energy Commission could result in a 10 per cent increase in the number of cases of cancer or leukaemia in the US, the AEC maintains that such estimates are grossly exaggerated. Dr T. J. Thompson, Commissioner of the AEC, recently stated that "the hazard from reactors to the population as a whole is at an extremely low level and is being badly distorted in the present overcharged climate of emotions". More specifically, he remarked that the increased exposure to radiation near the edge of a nuclear reactor site is equivalent to that gained by living on top of a 400 foot hill instead of at the bottom. This must be a very small amount compared with the natural background of radiation. Indeed, Dr Thompson said, there is probably statistically less than one extra case of cancer or leukaemia as a result of nuclear reactors. By comparison, the environmental pollution from coal fumes and smog causes very much more damage.

Notwithstanding such reassurance, "conservationist" opposition to the construction of nuclear power plants is slowing the building programme. This is not, however, the cause of the present brownouts and blackouts, which result from underestimates of power requirements which were made some five years or more ago, when many of the present power plants were designed and built. The present concern over environmental pollution is likely to result in insufficient provision of power facilities in 1975 and after. The AEC's concern over the slowing of the nuclear power plant building programme is reflected in continual tightening of the conditions for design and operation of nuclear reactors. One notable attempt to reassure public opinion about the safety of nuclear reactors is newspaper advertisements which point out that President Nixon's Western White House at San Clemente, California, is within striking distance of a nuclear reactor. If it is safe for the President, imply the advertisements, it is safe for everyone else.

Table 1. ELECTRIC POWER CAPACITY OF THE US

Year	1950	1969	1980 est.	2000 est.
Population	152	204	235	320 million
Total power capacity	85	313	668	1,352 million kW
Nuclear power capacity	0	4.3	150	941 million kW
Nuclear proportion	0	1	22	69 per cent

NEWS AND VIEWS

Mapping Human Genes

UNTIL the linkage groups and chromosomal locations of human genes have been determined human genetic engineering will remain little more than a pipe dream. That is not, of course, to say that ignorance of human somatic cell genetics is all that stands in the way of genetic engineering. Even if linkage groups assignable to every human chromosome were known today genetic engineering would not immediately become a practical proposition, but without such information any attempt to change purposively the genome of a human cell has little chance of success. It is not surprising, therefore, that the genetic analysis of human somatic cells is currently attracting a great deal of attention and that the experiments reported by Bodmer and his colleagues, on page 248, and Ruddle, Chapman, Chen and Klebe, on page 251, are so welcome.

These experiments strongly suggest that the structural genes for two human enzymes, peptidase B and the B subunit of lactate dehydrogenase, are linked and located on an autosomal chromosome. Moreover, both groups agree that the structural gene for the A subunit of lactate dehydrogenase is not linked with that specifying the B subunit, and apparently Bootsma and his colleagues in the Netherlands have reached this conclusion independently using a different experimental system. Although linkage between human genes on the sex chromosomes has been reported this seems to be the first example of a human autosomal linkage group, and the work is yet another testimony to the power of Watkins and Harris's technique for inducing the fusion of somatic cells by treating them with inactivated Sendai virus.

Both groups approached the problem of establishing gene linkage in the same way. They used Sendai virus to fuse human white blood cells with mouse cells of the stable cell lines 3T3 or RAG. The resultant human mouse hybrid cells preferentially shed human chromosomes as they are cultured. This, as yet unexplainable, phenomenon can be exploited to establish linkage between human genes simply by assaying a series of hybrid cell lines for human gene products, in this case enzymes, which can readily be detected by gel electrophoresis of cell extracts. Leaving aside any complications arising from regulator genes, chromosomal translocations and selection pressures for the retention of particular human chromosomes, if two human genes are linked and are on the same chromosome the enzymes they specify will only be present in hybrid cells retaining that chromosome. Once the chromosome is lost both enzymes should be lost simultaneously. So by correlating the enzyme activities of a series of hybrid lines it should be possible to establish gene/gene linkage, and by examining the karyotypes of the hybrids and analysing the number and type of human chromosomes they retain it should be possible to

decide which chromosome carries the linked genes.

It scarcely needs saying that experiments of this sort are extremely tedious in practice and fraught with pitfalls. Any translocation of chromosomal material can result in an apparent but spurious linkage. The instability of the karyotypes of human mouse hybrids and the occasional appearance in a hybrid line of a chromosome unlike those of their parent cell can cloud the issue. And if the expression of the structural genes being analysed is controlled by regulatory genes, which are unlinked, the interpretation of patterns of enzyme activity in hybrids can become hopelessly complex. Furthermore, if the particular murine and human enzymes have the same electrophoretic mobilities, as do murine and human lactate dehydrogenase B subunits, distinguishing them in cell extracts poses considerable problems.

Aware of all these snags both Bodmer and his colleagues and Ruddle *et al.* have analysed enough hybrid cell lines, clones and subclones to conclude that human lactate dehydrogenase B and peptidase B genes are linked. There is a positive correlation between the occurrence of the two enzymes; hybrid cells either have both or neither. And the combined data make it highly unlikely that the two enzymes are on different chromosomes which are always lost concomitantly, or that the structural gene for one enzyme is linked to a regulatory gene for the other. Moreover, according to Ruddle *et al.* analysis of the inheritance of the mutant form of lactate dehydrogenase B, in the family of the mother and daughter whose leucocytes they used to make hybrids, indicates that the two genes are located on an autosome. Neither group found any evidence of linkage between about a dozen other enzyme activities which they assayed in the hybrid cell extracts. Having identified this one gene linkage the next, and far more tedious, step is to identify the autosome which carries the two genes.

This will involve searching for correlations between the enzyme phenotypes and the complements of human chromosomes in a set of hybrid cell lines. Ruddle and his colleagues have made a start, identifying the human chromosomes in their hybrids by their morphology and their failure to hybridize mouse satellite DNA or its complementary RNA. Most of their clones apparently contain between ten and sixteen human chromosomes with representatives of each class. They have not yet identified the autosome which carries the lactate dehydrogenase B and peptidase B genes, but they have managed to confirm that the unlinked lactate dehydrogenase A gene is on a C group chromosome, possibly the X chromosome.

What of the future? These experiments establish that hybrid cells can be used to identify autosomal

linkage groups. As the number of antigens, enzymes and other genetic markers that can be readily assayed increases, the technique is bound to become more popular, and other linkage groups will soon be recognized. After that the problem will be to refine methods for the analysis of linkage within a particular chromosome. For example, it may be possible to do this by

analysing the phenotypes of cells containing chromosomes which have suffered deletion mutations of varying severity. As for medical exploitations, a dictionary of human linkage groups could immediately prove useful, in foetal medicine, for the early detection of genetic metabolic defects, or for the antigenic pre-selection of sperm before fertilization.

Chip Off the Old Block

No international committee likes to die a natural death, so the phoenix-like emergence of an International Geodynamics Commission to take over the running when the Upper Mantle Project comes to an end is hardly a surprise. By all accounts Professor Coulomb of Paris, who is bowing out of geopolitics after distinguished service, was concerned that a strong body should continue to exist, and an *ad hoc* committee has been at work in the past year trying to pinpoint the growth areas which needed emphasizing. As a result of this, a report was issued last July recommending a strong interdisciplinary approach to the problems of earth sciences in the '70s. The emphasis of this report is very firmly on the new tectonics approach and on the significance of lateral heterogeneity in all properties of the Earth in the outer 700 km. It is satisfactory that the report was constructed by relatively young earth scientists who have contributed much to our present understanding of the Earth, and the international example of letting the youngsters decide the programme is one that many national committees would do well to emulate.

The Commission has recently had its first meeting to produce an agreed statement and four needs were emphasized in these meetings.

(1) An increased understanding of the forces and processes between lithospheric blocks or plates.

(2) Studies aimed at understanding the driving forces on these blocks, involving *inter alia* laboratory studies at high pressures and temperatures.

(3) Some new ideas on the as yet little understood vertical movements within blocks.

(4) Palaeogeophysical studies to determine whether present day processes were equally valid in past geological time.

Partly from an obvious desire to relate global geophysics to human needs, the Commission appears to have placed hope in economic advantages from deep drilling programmes comparable with the present JOIDES project. A recent discovery of copper in a JOIDES hole may help their case.

The Commission's deliberations are notable both for what was included and what was omitted. The very strong emphasis on a block or plate approach to the earth sciences is most important. Some people who should know better seem woefully ignorant of the enormous implications of the revolution of the last three years. The Commission has clearly laid down

its belief in these new approaches and also in problem orientation rather than science for science's sake. Equally it has been careful to avoid giving any pats on the back to specific projects which might lead to its being used as a lever to extort financial support. Notably absent is any great enthusiasm for planetary exploitation, for local studies of "unusual regions" and for geotraverses (whether seismic, magnetic or gravity) —vastly expensive and largely useless operations that some countries have in the past committed themselves to.

So there is no recipe to national committees on what to support and what not to support, but a fairly clear indication of the direction in which the experts feel things should go. What exactly the Commission will succeed in doing remains to be seen. It has little power except persuasion and it is undoubtedly going to alienate many by being so forward-looking. The earth sciences are moving so fast that there are bound to be many who fall by the wayside, but on the other hand the quality of new recruits to the subject is encouraging. At first sight the Commission seems to be defining goals which are accessible to very few, but undoubtedly it will try to encourage all workers in the field to see national capabilities in a global programme. Whether Britain acts to implement financially work which is in accord with the aims of the Commission remains to be seen. Its record of official support for the Upper Mantle Project was not very inspiring despite the fine reports which were periodically distributed showing everyone apparently working on the Upper Mantle. There is clearly no reason why Britain should force earth scientists to follow the precepts of the Commission. On the other hand one hopes that there will be at least an awareness of the need to give extra help to those who take its recommendations seriously.

POLYNUCLEOTIDES

Looking at Coils

from our Molecular Biology Correspondent

It is clearly possible to leap into the deep end of molecular biology and survive, with no more knowledge about the physical properties of nucleic acids than what pairs with what. But to uncover the rules that govern the structures and interactions of nucleic acids it is necessary to study the properties of the ordered

and disordered forms individually and in depth. In spite of much work and some argument on the nature of the stacked state of unpaired polynucleotides, little thought has been given to the properties of the unstructured chain. This has now been rectified in a monumental study by Inners and Felsenfeld (*J. Mol. Biol.*, **50**, 373; 1970) on polyribouridylic acid. This polymer serves as an archetype for the disordered state, for in aqueous solutions its optical properties are consistent with the absence of any appreciable interaction between the bases.

As in the earlier work from the same laboratory on poly A, it proved possible by selection of ionic strength and temperature to find conditions of ideality, where the combined effects of polymer-polymer and polymer-solvent interactions effectively vanish. A precipitation method was devised for fractionating the poly U into cuts of narrow molecular weight range. The dependence of sedimentation coefficient and intrinsic viscosity on chain length obeyed the law required for a random coil. With changing temperature these parameters and also the radius of gyration, determined from light-scattering, which measures the flexibility of the coil, showed only a small and gradual change, by marked contrast with poly A, which displays large changes in physical properties, as the stacking interactions diminish. A quantitative expression that defines the flexibility of the coil can be derived by relating the radius of gyration to the number of monomer units in the chain, and the length of each. This expression turns out to be independent, as it should be, of the molecular weight, and its magnitude betokens a stiff chain, quite different from polyphosphoric acid, for example. It is equally, however, very different from stacked poly A, but essentially identical with unstacked (melted) poly A, so that the dimensions in solution of a disordered polynucleotide appear to be unrelated to the nature of the base. It follows from the value of the coil parameter that there must be extensive restrictions on the permitted rotational angles. Conversely, if one can guess at the values of the six angles of rotation that define the chain geometry, one can calculate the coil dimensions. Now crystallographers have noted that all the angles fall into narrow ranges for the many nucleotides and base-paired polynucleotides of known structure. Inners and Felsenfeld have shown that if these values apply also to poly U in solution, they lead to calculated coil dimensions that are entirely compatible with the experimental values. Not only this, but if the same computation is applied to poly A with introduction of stacking at random along the chain, the effect on the radius of gyration is again very like that actually observed. It remains only to determine why the restrictions on the freedom of the chain are so stringent in solution; one of the more likely contributing factors is that even at high salt concentration the repulsion between phosphates is sufficient to cause the distance between them to be maximized.

It may be noted that when the temperature falls to 10° C or below, an ordered form of poly U makes its appearance in a cooperative manner. Thrierr and Leng showed by light scattering, and Millar and Mackenzie (*Biochim. Biophys. Acta*, **204**, 82; 1970) now confirm by sedimentation equilibrium, that this transition is accompanied by no change in molecular weight, and must therefore be considered to involve the formation of hairpins folded back on themselves, or possibly more complex branched forms, such as are

formed in poly dAT at intermediate temperatures. In this connexion it is of interest also that a kinetic study has appeared (Yeh, *J. Chem. Phys.*, **52**, 6218; 1970), which depends on an interesting new technique that has been glittering brightly on the horizon. Diffusion coefficients can be determined from the wavelength distribution of scattered light, when a laser is used to provide a coherent incident beam: the doppler broadening of the scattered light then depends on the velocity of motion of the solute molecules. When, as here, two species of different hydrodynamic properties are in equilibrium, the method can in principle—and, it now appears, in practice, though various approximations remain to be properly evaluated—be used to extract the rates of interconversion from the line shapes, in a range extending to 10^7 s^{-1} . This could well become an important method for studying the kinetics of macromolecular processes.

TUMOUR VIROLOGY

Rapid Transformation

from our Cell Biology Correspondent

Baltimore and Temin's discovery of an RNA dependent DNA polymerase associated with the virions of tumour viruses (*Nature*, **226**, 1209; 1970) has, predictably, caused prompt reassessments of the research programmes of numerous laboratories. And by all accounts similar enzymes have been found in numerous RNA tumour viruses by virtually every group that has looked for them. Characterization of these enzymes and their DNA product is likely to remain a chief preoccupation for many months to come.

One of the more interesting questions to be answered is what is the fate of the DNA made by the enzyme? Is it integrated into the nuclear DNA of the host cell as Temin predicts? Hybridization experiments reported by Baluda and Nayak (*Proc. US Nat. Acad. Sci.*, **66**, 329; 1970) suggest that this will prove to be the case. They have measured the extent of hybridization between 71S RNA of avian myeloblastosis virus and DNA of leukaemic chick myeloblasts and normal chick embryo cells. In all, 1.7 times more viral RNA hybridizes to leukaemic cell DNA than to normal cell DNA and this difference is not simply the result of a differential but non-specific capacity of the two DNAs to bind RNA. Moreover, competition with a sixteen-fold excess of cold viral RNA reduces the binding of hot viral RNA to leukaemic cell DNA to the level obtained when normal cell DNA is used. Melting curves of the hybrids of the viral RNA and leukaemic and normal cell DNA show the respective values of T_m to be 92° and 87° C. Clearly, apparently normal chick cells contain sequences of at least 100 nucleotides complementary to the myeloblastosis viral RNA. Baluda and Nayak believe that this homology may indicate that the normal cells are latently infected with a tumour virus. Taken together, their results, although not completely conclusive, suggest that the DNA made from tumour virus RNA templates is integrated into the host cell DNA.

On page 245 of this issue of *Nature*, Hausen and Schulte-Holthausen report broadly similar hybridization experiments which reveal small amounts of EB virus DNA in a "virus-free" line of Burkitt tumour cells. This work, together with experiments of others which show that EB virus can transform cells in culture,

strengthens the suggestion that EB virus may be the causative agent of Burkitt's lymphoma.

Watson, Ralph, Sarkar and Cohn (*Proc. US Nat. Acad. Sci.*, **66**, 344; 1970) have reported experiments which raise the possibility that a leukaemia type virus is involved in the aetiology of mouse myeloma. They have found a positive correlation between the capacity of mouse myeloma cells in culture to synthesize immunoglobulins and to produce a murine leukaemia type virus. Using various tests, Watson *et al.* identified the virus-like particles seen in the myelomas and in their culture media as a virus closely related but not identical to known murine leukaemia viruses. The myeloma cells which produce virus carry the Gross leukaemia type G antigen and the viruses themselves have leukaemia group specific antigens. Moreover, Watson *et al.* have found that a variant line of myeloma cells, isolated by Schubert and Cohn in 1968, which fails to synthesize immunoglobulins also fails to produce virus. And some variant cells selected for lack of leukaemia antigens also failed to synthesize detectable amounts of immunoglobulin proteins.

DISEASE TRANSMISSION

Viruses on the Wind

from our Microbiology Correspondent

DURING the past few years, evidence has been accumulating to implicate climatic conditions as a major factor in the dissemination of foot and mouth disease. Last year (*Nature*, **223**, 712; 1969), Smith and Hugh-Jones reported that wind could be of primary importance, and now, two years after the last calamitous outbreaks in England and Wales, Hugh-Jones and Wright have published a study of the 1967-8 epidemic in relation to the then prevailing weather (*J. Hygiene*, **68**, 253; 1970). Although numerous agencies have been suggested as carriers of the virus—animals, carcasses, milk, bones, birds, rats, hay, vehicles and humans included—the cause of only 5 per cent of the outbreaks in 1967-8 was established conclusively. How then did most of the infections—more than 2,000—occur? Hugh-Jones and Wright's analyses show that wind and subsequent precipitation were strong contenders for the role of chief disseminating agents.

The first identified outbreak was on a farm at Nant Mawr, Shropshire, in October 1967. The pattern of the spread of the disease from this centre conformed to that predictable for windborne spread from a point source. The mean bearing of outbreaks in the invasion sector was 039°, while the concurrent mean surface and 2,000 foot wind directions were 034° and 068° respectively. Moreover, a calculation of the axis of a plume of windborne material in these conditions gave a value of 041°. Thus, the initial spread in the epidemic of 1967-8 was aerial and relatively close to the ground.

An investigation of a sub-epidemic near Worcester proved particularly interesting because of its isolated situation. Foot and mouth disease was introduced into this area in skim milk pig feed, but subsequent spread in the locality was by other means. Indeed, the subsequent pattern of outbreaks accorded closely with the weather conditions. Thus, there was little if any spread during a subsequent dry, anticyclonic period with little wind, although there were adequate numbers of susceptible stock on nearby farms. With the return of

rain, however, there were fresh incidences of the disease. As well as transmission of the virus for short distances by wind, cases of windborne infection for 60 to 110 km were detected. The data collected from the 1967-8 epidemic also point to an association between wind speed and rate of spread of the disease. In mid-November, for example, a sharp decrease in the rate of spread coincided with the onset of dry weather, whereas maximal rates of spread later in the winter correlated with periods of snow bearing winds.

If foot and mouth disease is transmitted by wind, several assumptions relating to the release of the virus into the air, its transportation and eventual deposition must be made. The virus can be emitted from infected animals in several ways, including sneezing, and probably appears in droplet nuclei in the air. The size of the droplets largely determines the extent of spread; strong winds which dehydrate and fragment large droplets lead to greater spread. One case history investigated in detail by Hugh-Jones and Wright argues forcibly for an aerosol transmission of foot and mouth disease. In concluding that wind is responsible for spread they are rather pessimistic about the chances of finding an effective means of containing the disease. Accurate weather forecasting coupled with early diagnosis should aid farmers and epidemiologists and enable them to alert and concentrate on areas of probable infection. Immediate slaughter of all infected and contact animals remains the chief method of choice for containing the disease in Britain.

IMMUNOCHEMISTRY

Eye on Antibodies

from a Correspondent

IMMUNOCHEMISTS from ten laboratories cross matched almost seventy antibodies to the proteins of the vertebrate eye lens when an international working party on crystallin immunochemistry met in the MRC Epigenetics Research Group, Edinburgh University, from June 23 to July 6. Additional antibodies are expected from three more laboratories. The lens of the vertebrate eye is often used to study problems of embryonic induction, tissue specificity and metaplasia, regulation of protein synthesis and protein evolution. Lens proteins, or crystallins as they are called, are also studied in cataract and other human pathological conditions. Because the crystallins are non-enzymatic, immunochemical procedures are used in their recognition. If antibodies of unknown differences in specificity are used discrepancies in the literature are to be expected, yet it has become increasingly necessary to be able to make detailed rather than very general correlations between data from various laboratories. To achieve this, the antibodies were cross matched both qualitatively and quantitatively in standard conditions against a panel of antigens.

The test panel included antibodies to total crystallins of fifteen species including man, bovine, chick and amphibia and antibodies to restricted fractions from several of these species. The panel of test antigens included total crystallins of fourteen species to fourteen different preparations of human, bovine, and chick crystallins fractionated by chromatography or isoelectric focusing and ten highly purified bovine crystallin fractions, including crystallin subunits.

Four types of antibodies will be designated and reserved in a reference collection: (1) antibodies detecting the maximum number of antigenic constituents in a specified antigenic preparation, which may be used as a reference to compare antibodies from different laboratories with respect to the antigens recognized; (2) antibodies specific to a class of crystallins within a species; (3) antibodies which discriminate between members of a class of crystallins; and (4) antibodies which discriminate between antigenic determinants in highly purified preparations. The last two types include species cross-reactive antibodies.

The results of the cross matching will also be examined to determine the number of antigenic determinants detectable within a class of crystallins of a given species, and compare the antigenic composition of a range of species.

Plans were also made for exchanges of antibodies and purified antigenic preparations between biochemists and immunologists. This will make possible both the detection of minor impurities in protein preparations and the production of antibodies against specified constituents.

A full report of antibody specificities and standards and other findings will be published, together with a list of participants and acknowledgments to the organizations which financed the working party, the firms which helped with loans of apparatus and the biochemists who donated purified crystallin preparations. Pending the collation and publication of results any investigators who have anti-crystallin antisera which they would like to have cross matched against the present panel of reagents are invited to communicate with Mrs R. Clayton at the Institute of Animal Genetics, Edinburgh University.

CROPS

Rice Races Raised

WORLD records for the annual production of rice have been claimed by experts at the International Rice Research Institute in the Philippines. Dr S. K. De Datta writes: Three crops were harvested between January 1969 and January 1970 at the experimental farms of the institute, in Los Baños, and of the Central Mindanao State University. At Mindanao the highest total yield came from a transplanted indica line, IR593-3-17, which produced 23,553 kg/hectare in 315 days (Table 1). As far as we know this is the highest authenticated annual production recorded from a single variety or line. The highest yield previously reported for three crops of transplanted rice was obtained at Ivoloina Experiment Station in Madagascar, when a japonica variety, 'Chianan 8', produced 20,430 kg/hectare in 335 days (J. Velley, *L'Agronomie Tropicale*, No. 8, 669; 1969).

At Mindanao IR20 and the well known dwarf variety IR8 also gave very high yields (Table 1). IR20, a variety newly released from the institute, produced 23,226 kg/hectare in three crops in 300 days; IR8 produced 22,826 kg/hectare in 316 days. When the total yield was computed by adding the three best yields the annual production was 24,279 kg/hectare; our previous record was 20,174 kg/hectare (*International Rice Research Institute Annual Report*, 1966). This is a reasonable calculation to make because many

Table 1. YIELDS OF THREE CROPS OF TRANSPLANTED RICE IN MINDANAO IN 1969-1970

Variety or line	Crop	Yield of grain (kg/hectare)
IR8	Jan. 16 to May 6	10,081
	May 21 to Sept. 3	6,133
	Sept. 11 to Dec. 21	6,612
		22,826
IR20	Jan. 16 to Apr. 25	9,392
	May 21 to Sept. 4	6,828
	Sept. 11 to Dec. 15	7,006
		23,226
IR593-3-17	Jan. 16 to May 4	10,274
	May 21 to Sept. 3	6,102
	Sept. 11 to Dec. 22	7,177
		23,553

Each crop received 60 kg/hectare each of P_2O_5 and K_2O . For each variety and line nitrogen was applied as 140 kg/hectare to the first of the three crops, 120 kg/hectare in the second and 80 kg/hectare to the third.

farmers who grow three crops in a year use three different varieties.

In our experiment at least 49 days were available to prepare the land for the three crops, an important consideration for the farmer. It is relatively easy to grow three crops with, say, 120 day maturity within 365 days. But this leaves only 5 days for the preparation of the land, which is not enough in real farming conditions. If, however, at least 10 days are available between each crop, three crops a year are within the reach of good farmers.

Table 2. YIELDS OF BROADCAST IR8 AT LOS BAÑOS DURING 1969-1970

Crop	Yield of grain (kg/hectare)
Jan. 8 to May 7	8,840
May 13 to Sept. 2	5,525
Sept. 11 to Jan. 2	6,718
	21,083

Nitrogen was applied as in Table 1.

At the institute's farm, IR8 gave the highest yield—21,083 kg/hectare in 365 days—after being grown from seed rather than from transplanted rice (Table 2). In spite of this success it is not likely that the use of broadcast seed will be adopted widely in Asia in the near future, for it will require a controlled water supply, which the monsoon season hardly provides, and very careful management if such high yields are to be repeated.

PLANT BREEDING

More Room for Growth

by our Botany Correspondent

THE gates of the Plant Breeding Institute in Cambridge were opened on July 9 to a bevy of inquisitive visitors who gathered for the opening of a much needed new block of laboratories. Called the Douglas Bell Building,

it is named in honour of Dr G. D. H. Bell, who has been director of the institute since 1948 and saw it set up on its present site in 1950. The prestige that plant breeding has attained during the history of the institute was underlined by the presence at last week's ceremony of the President of the Royal Society, Lord Blackett, who admitted that he had little contact with plant breeders during his youth in the Cavendish Laboratory.

While wandering through the laboratories and glass-houses, or riding by tractor around the extensive fields of experimental material, visitors could see clearly how the institute holds to its dual function of producing new varieties of arable and herbage crops, and promoting pure and applied research into many aspects of plant breeding. One of the teams that has moved into the new building is the cytogenetics group under Dr R. Riley, who has been appointed to take over the directorship of the institute when Dr Bell retires next year. Apart from the well known work on wheat, exploiting the genetic control of chromosome behaviour, there is a developing programme in barley cytogenetics. This is aimed at achieving the duplication of loci that will be useful in conferring disease resistance.

The biochemical genetics programme, which is to be concerned with phenomena of the nucleus and other organelles which are significant in crop genetics, has already produced some interesting results. Dr M. D. Bennet has found that for diploids the duration of meiosis is proportional to the amount of DNA in a cell. Although known already for mitosis this is the first time such a relationship has been identified in meiosis.

Bennet also thinks that he has found a possible reason for the instability of *Triticale*, the hybrid from wheat, *Triticum aestivum*, and rye, *Secale cereale*. Measuring the duration of meiosis, he obtained values of 24 hours for *Triticum*, 20.75 hours for *Triticale* (the fastest known plant in meiosis) and 51.2 hours for *Secale*. The discrepancy between the parents suggests that asynchrony of cell division is the cause of instability in the hybrid. This could be a very significant finding if it were the key to the problem of producing the highly desirable stable hybrids which would combine the useful features of *Triticum* and *Secale*.

One of the chief concerns of the plant breeders at the institute is the development of strains resistant to disease. One quite new programme—in progress for two years—is the search for sugar beet which is resistant to aphids. Dr H. J. B. Lowe has found that different clones of aphids prefer to live on different concentrations of sugar, and he suspects that resistance in this case depends partly on the concentration of sugar in the plants. Other substances such as amino-acids seem to be involved in a similar way.

With other crops progress has, of course, been more extensive, and visitors were shown a list of eleven varieties of various species developed at the institute and currently recommended by the National Institute of Agricultural Botany (NIAB). One of the most exciting of recent developments, the winter wheat Maris Beacon, has outstripped all rivals in yield during three years of trials at NIAB. Unfortunately yellow rust, developed last year, has delayed marketing of this variety. If it proves unduly susceptible to the disease it is hoped that a sister variety, Maris Nimrod, with equivalent yield, will prove resistant.

SLIME MOULDS

Further Cause for Attraction

from our Microbiology Correspondent

BONNER and his collaborators at Princeton report that they have distinguished a second chemotactic system in the slime mould *Dictyostelium discoideum* which has the distinctive property of stimulating vegetative amoebae. Describing their findings in the *Journal of Bacteriology* (102, 682; 1970) Bonner, Hall, Sachsenmaier and Walker report how an unknown chemotactic substance is produced by bacteria, that is if *Escherichia coli* can be taken as typical in this context. The conclusion of major significance is that this newly discovered agent and acrasin (cyclic-3',5' adenosine monophosphate) exert stage-specific responses on the developing amoebae.

Chemotaxis was tested by the 'Cellophane' square method devised some years ago in Bonner's laboratory. Vegetative amoebae are allowed to settle on small squares of washed 'Cellophane' which are then placed on agar containing the test substance. The test is read by recording the rate at which the amoebae move away from the 'Cellophane'. For acrasin, it is known that the movement of the amoebae is directed by a concentration gradient that is produced and maintained through the action of a specific phosphodiesterase (*Nature*, 223, 672; 1969).

Bonner *et al.* applied this test to cyclic-AMP and aqueous extracts of *E. coli* B/r. The mean rate of movement induced by a bacterial extract was significantly greater than that induced by 1.5×10^{-5} M cyclic-AMP. Furthermore, the chemotactic ratio, defined as the distance moved towards or away from a source over the total distance travelled, indicated that crude bacterial extracts had very strong orientation properties. Cyclic-AMP had a maximum chemotactic effect for concentrations of 10^{-5} to 10^{-8} M.

Bonner *et al.* argue that because the bacterial extracts produced a much greater degree of orientation, a chemotactic substance additional to cyclic-AMP was present. The new substance was demonstrated by another means. Being unable to diffuse through agar gels, the bacterial extracts freed of cyclic-AMP elicited no attraction when placed adjacent to small populations of vegetative amoebae. But such amoebae were attracted away from original populations when the dialysed extract was mixed into the agar. A concentration gradient of the new chemotactic substance is maintained, like cyclic-AMP, by secretion of an inactivating enzyme by the vegetative amoebae. Thus, dialysed bacterial extracts lost all activity within thirty minutes when incubated with the crude, extra-cellular amoebal enzyme.

The 'Cellophane' square test was applied to vegetative amoebae—those still feeding—and to sensitive amoebae on the point of aggregating, using cyclic-AMP and dialysed bacterial extract. With cyclic-AMP, the sensitive cells aggregated in large waves but vegetative cells responded poorly. The chemotactic peak of cyclic-AMP for sensitive cells was very sharp, with a maximum at 10^{-7} M. Similar experiments with the bacterial extract produced a very different reaction; the vegetative cells had a strong positive response while the aggregating cells did not leave the 'Cellophane' square. Thus there seems to be a switch in chemotactic response during the development of the amoebae.

Perhaps the newly detected substance is a prime means of food seeking (it is usually abundant during the vegetative stage when bacteria are present), whereas cyclic-AMP acts specifically in the aggregation process. The identity of the new substance will be keenly awaited. Its non-diffusibility does not especially worry Bonner and his colleagues; such a feature may have little significance in nature where soil and humus are the normal substrates.

TRANSFORM FAULTS

A Job for Coriolis

from our Geomagnetism Correspondent

IN spite of all that has been written about mid-oceanic ridges in general, and about transform faults in particular, no one seems to have given a really satisfactory explanation of why the faults are there. In his original description of transform faults, Tuzo Wilson supposed that the offsets were inherited from the shape of the break which first formed between the coasts of the adjacent continents—but really that just begs the question. And the question is quite important because, as Howell points out (*J. Geophys. Res.*, **75**, 2769; 1970), it is difficult to reconcile the offsets with a smoothly flowing convective system in the mantle. The sea floor spreads normally from each ridge section, but because each section lies at a small angle to the overall trend of the ridge the upwelling crest of any convective flow must be discontinuous.

Howell has come up with an ingenious explanation of how the bulk convective flow can be continuous and yet still be consistent with offsets. He suggests that it is all due to the Coriolis force, which causes the surface of the convection cell to drift sideways relative to the underlying flow. There is a well established precedent for such a phenomenon in the circulation of oceans and atmospheres. In the oceans, for example, a current at one level produces a flow at an adjoining level which is rotated clockwise in the northern hemisphere and anticlockwise in the southern hemisphere. In this case the effect can be so great that the net flow is normal to the surface driving force.

An obvious way to test Howell's hypothesis is to determine the sense of apparent rotation of the ridge sections relative to the overall trend. It turns out that more than two-thirds of the displacements are in the sense required by the Coriolis effect. Although this is a large enough difference to support Howell's idea, it suggests that Coriolis force is not the whole explanation. A second test derives from the variation of Coriolis force with latitude. Because the force is proportional to the sine of latitude, fault displacements should tend to be larger at high latitudes than near the equator. According to Howell, the total displacement along transform faults between 0° and 30° latitude is 61.4 arbitrary units, compared with 72.5 such units for faults between 30° and 60°. Unfortunately there are too few data above 60° to enable the comparison to be extended.

Hitherto, little attention has been given to the effect of the Coriolis force on mantle convection because for slow movements it is small. Runcorn, for example (*J. Geophys. Res.*, **69**, 4389; 1964), estimated that the Coriolis force was only 10^{-8} as large as the other forces involved. But the limits of error on this estimate are

certainly several orders of magnitude because neither the size of the convection cells nor the viscosity of the mantle material was known. Furthermore, there is no simple relationship between the magnitude of the forces and the amount of rotation they produce. Much depends on the coupling between the driving force and the mantle material and the thickness and viscosity of the layers through which the rotation is distributed. For these reasons it is impossible to give a realistic quantitative estimate of the effect of Coriolis force and thus definitely to rule it out. On the contrary, Howell shows experimentally that the Coriolis force is involved in some way. But because of its small size it is not surprising that it is not completely dominant.

AMORPHOUS SOLIDS

Shapely Currents in Carbon

by our Solid State Physics Correspondent

ELECTRICAL conduction in amorphous carbon seems to occur by a rich variety of processes, according to experiments by C. J. Adkins, S. M. Freake and E. M. Hamilton on thin films of carbon held in different conditions (*Phil. Mag.*, **22**, 183; 1970). At low applied fields, conduction along the films was found to be by a hopping process characterized by a special temperature variation with voltage, whereas at higher fields the mechanism was of the Poole-Frenkel type with a range of traps and a large effective dielectric constant. Conduction through the film was found to be appreciably poorer than along it, which was attributed to structural anisotropy in the films.

Within the past two or three years there has been a considerable upsurge of interest in the electrical properties of amorphous materials, and a recent review by a panel of the Science Research Council gave every indication that this interest will continue to flourish (*Nature*, **227**, 108; 1970). Carbon is a particularly suitable material to study in thin film form as the amorphous character of these films is strong. Electron microscope examinations suggest that the ordered structure is limited to 30 Å or less and that the crystallite size is typically no more than 10 Å.

Extremely large variations of resistance were found between different specimens, supposedly caused by variations in grain size and impurity content. The dependence of the resistance on temperature, however, at low applied fields and low temperatures was the same for each specimen, and followed the pattern predicted by Mott in which there is sufficient overlap of the localized states for tunnelling to occur beyond the nearest localized site.

At higher fields, the variation of current with applied field and temperature suggested to Adkins *et al.* the presence of Poole-Frenkel conduction. The parameter which denotes the trap depth turned out to be quite well defined above about 10 K, but decreased rapidly at lower temperatures. They point out that the drop in trap depth is not surprising as the extreme disorder implies a spectrum of depths of which only the most shallow would remain active at low temperatures.

They found that conduction through the film at low fields defied any simple explanation. The chief surprise was the flattening off of the resistance at 5 K as the temperature was lowered.

Relationships between Controllers of a System

by

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Many different arrangements of controllers can be designed to ensure the efficient functioning of a system. Whether the system is a complex piece of machinery or a committee in session, the principles are the same.

A SYSTEM with a control mechanism works satisfactorily as long as this mechanism does not deteriorate or break down. By "satisfactory" I mean that the behaviour of the system and its performance adhere to a set of standards; whether these are optimal or not is a different problem. Once the standards are defined and the control mechanism constructed, the system is expected to react whenever the standards are violated.

Control mechanisms, however, are susceptible to wear and failure, and when they cease to operate, performance may be substandard. For example, if an electric water heater is controlled by a thermostat which fails, the heater continues to heat the water well above the permissible temperature. A managerial control system is particularly prone to wear and tear: instructions may be ignored or circumvented and procedures misinterpreted or forgotten. Soon an intricate and polished communication and control system is no longer fully effective.

There are three possible methods of guarding against this. The first is to use several identical control mechanisms to operate in concert. "Identical" means here that the mechanisms are designed to operate and react to the same conditions and stimuli. Their internal structure and design need not be the same, but any variations in behaviour are attributed to chance variations.

The second method is to use non-identical controllers. These react to different sets of stimuli. The purpose of linking identical or non-identical control mechanisms is to provide an opportunity for the controllers to help each other in protecting the system from some specified undesirable breakdowns.

The third method is to check, continuously or occasionally, the performance of the control mechanism in relation to the performance of the system and the circumstances in which it operates. For example, it may be necessary to test whether a production controller continues to adjust the production level when he should, or to determine whether the stimuli that activate the controller are adequate to deal with the situation, and whether control should be transferred to another controller better equipped to react to changes in the system. Such an arrangement that calls for the controller's performance to be under scrutiny can be termed "control of control".

Linkages between Multi-controllers

The linking of several identical controllers is called first order control, and is characterized by all the con-

trollers having equal status and opportunity to affect the state of the system. This does not happen when non-identical controllers are linked, because these are designed to react to different stimuli, and so may not have equal opportunity to react. In some cases the linking of two non-identical controllers leads to second order control, in which one controller acts most of the time and the other only some of the time, or when the first controller fails.

I shall discuss three types of control linkage: control in series, control in parallel and conjoint control. These are significant in the way they can impose—or are prevented from imposing—their will on the system, and in the way they affect the probability of breakdown of the system.

Table 1 shows the way in which a controller that can assume two states, ON or OFF, actually responds compared with the way he should respond. There are two types of failure: type 1, failure to switch ON when required, and type 2, failure to switch OFF when required. When considering the effect of the control procedure on the probability of failure it is important to remember the distinction between these two types of failure, for each may be affected in a different way by the control linkage that is adopted.

Table 1. TYPES OF FAILURE TO RESPOND

The way controller(s) should respond	Controller(s) actual response	
	ON	OFF
On	No failure	Failure 1
Off	Failure 2	No failure

First Order Controllers in Series

An example of first order controllers in series is provided by an electric boiler with its temperature controlled by two identical thermostats connected in series (Fig. 1a). If both are working properly and switched on and off at the same temperature, the control procedure is just as effective with one perfect control mechanism. But if one thermostat fails to switch OFF, the electric circuit is broken by the other, and vice versa.

But the performance of the boiler may well depend on the type of failure of the thermostats. As long as this failure occurs in switching OFF, the reaction of one thermostat effectively compensates for the sluggishness or complete breakdown of the other. But if both thermostats are at the OFF position and the temperature declines

to the point where the current should be switched on again, and if one thermostat now fails to react, the system will remain OFF whatever the other thermostat does.

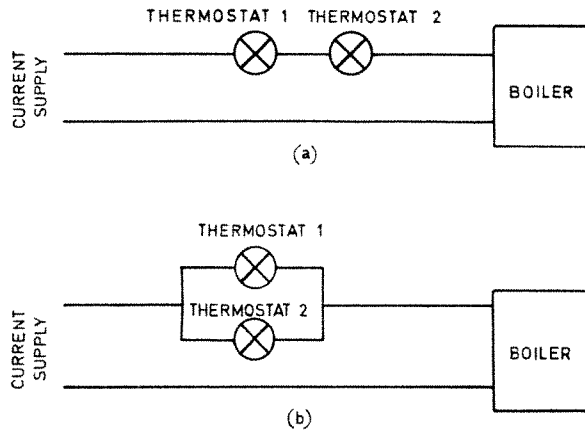


Fig. 1. Two control linkages. (a) Control in series; (b) control in parallel.

For control to be effective in this case, therefore, it is sufficient for only one thermostat to switch OFF but essential that both switch ON. If the probability of failure to switch OFF when required is p_0 for each thermostat and if p_1 is the probability of failure for each to switch ON, then the operation of the two-thermostat system is described by the probabilities in Table 2. The probability that the system is not switched OFF when it should be is $P_0 = p_0^2$, whereas the probability that it is not switched ON consists of the probability that thermostat 1 fails when 2 does not, $p_1(1-p_1)$; the probability that thermostat 2 fails when 1 does not, $p_1(1-p_1)$; and the probability that both fail, p_1^2 . In other words, the probability P_1 that the system is not switched ON when it should is

$$P_1 = p_1^2 + 2p_1(1-p_1) = p_1(2-p_1)$$

When p_1 is sufficiently small (say, less than 0.1), p_1 in the parentheses is negligible and the probability of failure to switch ON is approximately $2p_1$.

Table 2. FAILURE OF TWO THERMOSTATS

Thermostat 2		Thermostat 1	
		Need to switch OFF	Need to switch ON
		Failure p_0	Function- ing $1-p_0$
		Failure p_1	Function- ing $1-p_1$
Need to switch OFF	failure	p_0^2	$p_0(1-p_0)$
	functioning	$p_0(1-p_0)$	$(1-p_0)^2$
Need to switch ON	failure	p_1^2	$p_1(1-p_1)$
	functioning	$p_1(1-p_1)$	$(1-p_1)^2$

Thus the introduction of a second control mechanism provides an excellent insurance against overheating, but sometimes coupled with almost double the chance that the system will cease to operate (Fig. 2). In the case of a water-boiler this may be justified, but when it is undesirable that a complete shutdown is so likely to occur, control mechanisms may have to be redesigned to reduce p_1 , and with a moderate increase in p_0 the overall effect is generally beneficial. This applies to any system in which flow is controlled by mechanisms coupled in series. If the

mechanisms are valves which adjust the rate of flow of a liquid to any level up or down the scale, and not necessarily only to ON-OFF positions as in the thermostat, the governing factor is always the valve that allows the lowest rate. Failure to decrease the flow p_0^n for n valves and failure to increase it is $1 - (1-p_1)^n$ and when p_1 and n are sufficiently small this expression is approximately equivalent to np_1 .

The administrative analogy to the control system described here is a chain of executives, who function in sequence with relation to any set of operations they control. Consider the case where expenditure needs the authorization of two executives, both equal in status and unaffected by the behaviour of the other. Control is in series when authorization is conditional on both executives affirming their signatures on a requisition form (that is, both must be at the ON position), while only one needs to object to stop the expenditure (this executive being at the OFF position). The same procedure is prevalent with a cheque which requires two signatures: it can be cashed when the two signatures are ON; it is worthless if any one signature is OFF.

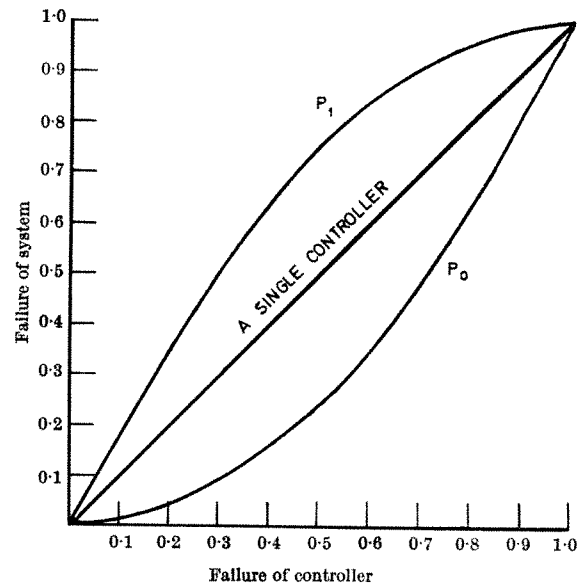


Fig. 2. Failure of system to switch OFF (P_0) or ON (P_1) for two controllers in series.

Another administrative example is provided by a flow of progress reports which is subjected to the scrutiny of several executives. Each may pick on an item in the reports and start an inquiry or issue instructions for modifications, or stop production. He can be said to be acting in a manner analogous to the thermostat that switches the circuit off. All the executives must acquiesce or raise no questions (or not read the reports) for no action to be taken, and this is when they are said to be at the ON position. But there is a significant difference between this situation and the case of the cheque: here ON is indicated by the *absence of reaction* on the part of the executive, whereas with the cheque, ON requires his *active response*.

Using the multi-thermostat analogy I propose the following definition for the administrative system. In a system in which control is exercised by the intensity of a signal or by two types of action, several control mechan-

isms are said to be operating in series if one action is triggered off by at least one mechanism, but the other action is enforced by all the mechanisms, and these actions of the mechanisms may be called OFF and ON respectively. The terms ON and OFF are used for convenience; they need not literally convey the connotation of switching operations. In the adjustment of, say, a production level, these activities may relate to pushing the level upwards or downwards, but by my definition "upward" indicates ON only if all control mechanisms are in agreement. If a procedure is set up whereby an increase in production rate is effected by one controller only, and a decrease by all controllers, then the first is equivalent to OFF and the latter to ON.

Controllers in Parallel

When controllers are connected in parallel, each can "activate" the system alone. In contrast to a linkage in series, where all the controllers need to be ON for the system to be ON but only one controller needs to be OFF for the system to be OFF, a linkage in parallel requires only one controller to be ON for the system to be ON but all the controllers have to be OFF for the system to be OFF. Switches in electrical circuits can be wired in series or parallel, and the type of linkage adopted has the implications that I have just described as to whether unanimity of agreement between the controllers is required for the system to change its mode of activity.

Table 3. SUMMARY OF PROBABILITY OF FAILURE

	Type 1 (Failure to switch ON) P_1	Type 2 (Failure to switch OFF) P_0
One controller	p_1	p_0
Two controllers in series	$p_1(2-p_1)$	p_0^2
Two controllers in parallel	p_1^2	$p_0(2-p_0)$

In the case of a water boiler controlled by two identical thermostats connected in parallel (Fig. 1b), if the probability of failure of either thermostat to switch OFF when it should is p_0 and its failure to switch ON is p_1 , then the probability of failure of both is given in Table 1. But because both thermostats have to act to switch the boiler OFF, the probability of failure for the system to switch OFF is

$$P_0 = p_0(2 - p_0)$$

whereas the probability of failure to switch ON is

$$P_1 = p_1^2$$

The position is therefore reversed (Table 3). If in Fig. 2 the graphs labelled P_0 and P_1 are relabelled P_1 and P_0 respectively the probability of failure for the linkage in parallel is obtained. Clearly control in parallel achieves the opposite effect of control in series—it provides a better protection from failure (compared with the single controller) to switch ON, but a poorer protection from failure to switch OFF. Thus the choice between the two control procedures may depend on the type of failure considered to be the most desirable to avoid.

For example, if when authorizing payment (or expenditure) two executives are assigned to handle the relevant documents and if it is thought that each should be allowed to authorize payment on his own, they are linked in parallel. When it is necessary to ensure a closer scrutiny

of the documents (particularly when large sums of money are involved) and reduce the risk of authorization being made by mistake, both executives may have to agree to the expenditure; they are then linked in series. But as I have shown, improved protection achieved in this way is coupled with an increased risk that managers will be over-cautious and fail to authorize justified expenditure.

Transactions at counters (such as payment of bills or purchase of merchandise) provide another example of a choice between linkage in series and parallel. When the volume of business necessitates several counters, the transactions are often organized as control in parallel. A transaction is offered by a customer to the controller manning the counter and, if the controller approves, the transaction goes through; if he is linked in parallel to the other controllers at other counters, he need not consult any of them. If the customer is dissatisfied with the decision, he can try another counter. Only when all controllers that are connected in parallel reject the customer does the transaction not take place. The implication in choosing this type of control for counters is that the penalty for type 2 failure (Table 1) is small, compared with the need to provide prompt service, and therefore the increased risk of incurring type 2 failures by introducing control in parallel can be tolerated.

A third example of a choice between linkages is provided by components that have to be inspected after leaving a production line. If the volume of production is large, and if the utility of a component is not excessive (for example, its monetary value or its future function), inspectors are often linked in parallel. As in the previous example, each inspector can "pass" the components; but if a component fails to pass one inspector, it can be sent to another (or even back to the same inspector) and there is, of course, a chance that it will pass the second time. Operators and inspectors know that this procedure increases the probability of components passing through when perhaps they should not, but in most cases the alternative of control in series is considered too costly and time consuming.

Conjoint Control

When control mechanisms are linked in series, it is sufficient for one to be at the OFF position for all the others to be neutralized. (This is reminiscent of the power of veto; members of a committee who have this power can be said to act as controllers in series, and any one member can prevent the others from taking action.)

To enable each controller to activate the system without the acquiescence of the others, controllers have to be connected in parallel, as I have described, but an activity cannot then be stopped without the agreement of all the controllers. And so control in either series or parallel requires the collaboration of the controllers.

A mode of control that allows each controller to act on his own, irrespective of whether activity needs to be started or stopped, can be called "assertive controllers in parallel", or better "conjoint controllers". In this case whenever one controller acts, it is as if the others automatically acquiesce with his decision and all controllers act together.

A lighting circuit with two switches has two conjoint controllers: each switch can switch the light ON or OFF on its own, irrespective of the state of the other switch. It is no longer necessary for both switches to act for the

light to be on and each switch can be regarded as automatically activating the other; when one goes on it overrides the other, and vice versa. The advantage is obvious: the system can continue to operate even if one controller breaks down. Unlike the system with several controllers in series or in parallel, which continues to operate for one type of breakdown but fails with another, a system with conjoint controllers will function as long as at least one controller is operational.

The distinction between the three types of control linkages discussed here is not solely characterized by the way information flows in the system, but also by the way controllers can affect each other. In administrative systems information may flow (documents or data) from one executive's tray to another, and in this case there may be a delay between decisions, and controllers further along the line may know how previous controllers have acted, or failed to act, in given situations. This type of information flow requires the controllers to act in sequence, and I have discussed the significance of such a mode of control before¹.

In the other form of information flow data are presented to the various controllers simultaneously, or at least when a controller is faced with the problem of making a decision, he does so without any previous knowledge of the decisions made by his fellow controllers. Thus, even if the information is not presented to all of them simultaneously, and if the decisions are not made at the same moment, the fact that each controller makes his decision on his own (he may speculate as to how the others have or will react, but he does not know for sure) means that this case can be treated as if controllers make their decisions simultaneously.

An important distinction between the three control procedures in the case of simultaneous decisions is that when linkage is in series or parallel each controller acts on the system but not on the other controllers, whereas in conjoint control each controller's actions automatically lead the others to acquiesce. In the first two, disagreement between controllers or failure of one can cause the system to get out of control (for example, shut down); in the third, disagreement manifests itself in the fact that each controller can always have the last word.

In each of the three procedures controllers have equal status. Each can affect the other in the same way, although one can prove to be more of a nuisance to the others in his actions, or failure to act when he should. Nevertheless, no controller dominates the scene or is able to dictate to his fellows, irrespective of prevailing circumstances. Even in conjoint control, when any controller can overrule the others, his action holds only as long as the others agree with him, otherwise the effect of his decision can be annulled by a counter-decision of another controller. Conjoint control does not have a power of veto vested in the individual controllers, as with control in series or in parallel. The power that each controller has to affect the state of the system and the limitations that his fellow controllers can impose on him are very different in the various control procedures. Nevertheless, when decisions are made simultaneously all controllers have an equal opportunity to affect the outcome for the system and it is in this sense that they have equal status.

Second Order Controllers

So far I have been concerned with the coupling of essentially identical control mechanisms, namely, mechanisms

designed to react to the same stimuli. The purpose of more than one controller, as I have shown, is to provide better protection against some kind of breakdown than is possible with one controller. Another way to achieve this is to link non-identical control mechanisms designed to react to different feedback signals regarding the performance of the system. In this way, two controllers can be set to respond with different frequencies, one looking after the system most of the time, while the other is required to act only when the need arises. This is second order control.

The principal difference, therefore, between several controllers acting within a first order or a second order procedure lies in the frequency with which the second mechanism needs to operate. If, for example, a mechanism is known to deteriorate the more it is activated, perhaps because of wear of its component parts, then the second order procedure may be advantageous. If, however, both mechanisms deteriorate with time, irrespective of how frequently they switch ON and OFF, the first order arrangement has the advantage of providing a better control (say, of a temperature level within closer limits) without loss of time-dependent reliability.

It should be pointed out that the type of failure of any one mechanism is just as important in a second order arrangement as in a first order arrangement. In other words, control can continue to be exercised when one mechanism fails to switch OFF; if either remains at the OFF position and fails to switch ON, the whole system is put out of action. The probabilities for failure (Table 3) are therefore equally applicable to a second order control system.

Second Order Control in Series

If two identical thermostats are linked to control the temperature of the water in a boiler, both are expected to react at the same time, but if the two are coupled so that one is set to react at two given temperatures and the other is set at more liberal levels (that is, the range between the two levels of the latter includes the two levels of the former), then the second thermostat only has to react when the first fails, and a second order control is established. In Fig. 3, thermostat 1 is designed to switch off at temperature L_1 and to switch on at l_1 , while thermostat 2 is designed to react similarly at L_2 and l_2 respectively.

In Fig. 3a and b the relationship between these control temperatures is

$$L_2 > L_1 > l_1 > l_2$$

In Fig. 3a thermostat 1 is master of the situation for a while, during which thermostat 2 is switched ON. Then at one point thermostat 1 fails to switch OFF when the temperature reaches L_1 , so that when it reaches L_2 thermostat 2 intervenes, and as long as thermostat 1 is out of action, thermostat 2 acts as a first-line controller, except that the temperature now fluctuates between l_2 and L_2 , rather than between l_1 and L_1 as before. Thermostat 1 later returns to action and reacts at L_1 and thermostat 2 assumes a passive role, until required again. But this depends on the kind of failure thermostat 1 is subjected to. In Fig. 3a it fails to switch OFF and so allows thermostat 2 to take over. In b, however, it fails at one stage to switch ON and when the temperature eventually falls to l_2 thermostat 2 cannot save the situation because 2 is ON

in any case and the linkage in series requires both to be on for an electric current to flow to the boiler.

In Fig. 3c the control levels are arranged as $L_2 > L_1 > l_2 > l_1$ and the ranges overlap, but the control procedure is unaffected. Thermostat 1 acts as a first-line controller for a while and the temperature fluctuates between l_1 and L_1 . At point 1 thermostat 1 fails to switch off and

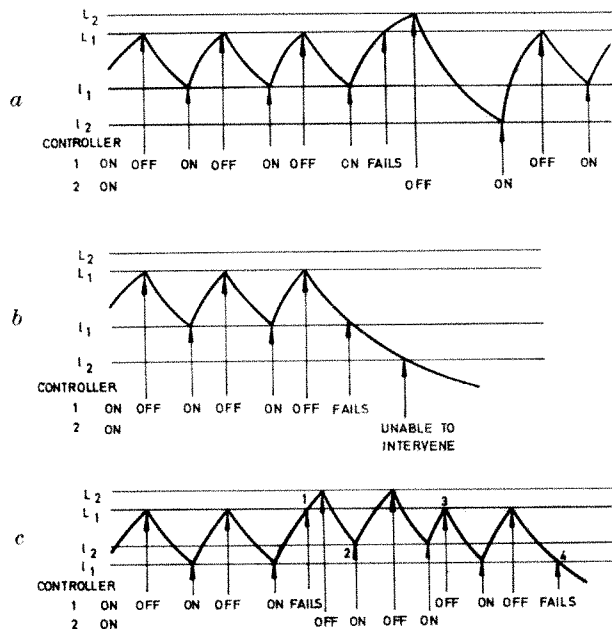


Fig. 3. Second order control in series.

thermostat 2 takes over and switches OFF at L_2 . When the temperature declines to l_2 thermostat 2 (point 2) switches ON and continues to act as long as thermostat 1 is out of action, with the temperature now fluctuating between l_2 and L_2 . At point 3 thermostat 1 is again operational as a first-line controller, but when it fails to switch ON at point 4 thermostat 2 cannot intervene and the temperature continues to decline below the desirable limit. There is, then, essentially no difference between the case where the control levels do not overlap (Fig. 1a and b), and the case where they do (Fig. 3c).

Second Order Control in Parallel

The effect of linking the two thermostats in parallel is shown in Fig. 4. The case $L_2 > L_1 > l_1 > l_2$ is shown in a and b. In a the first order thermostat is in operation, while the other thermostat is off. Then at one point the first order controller fails to switch ON and when the temperature declines to l_2 the second thermostat is activated. As in a linkage in series, the temperature fluctuates between l_1 and L_1 when the first order thermostat is in control and between l_2 and L_2 when the second order thermostat takes over. In Fig. 3c the control levels are $L_1 > L_2 > l_1 > l_2$ and, again, as long as controller 1 is operational, the temperature fluctuates between l_1 and L_1 , but when controller 2 starts to act the fluctuations are between l_2 and L_2 .

The difference between the linkage in series and the linkage in parallel lies, as I have shown, in the type of

failure of the first order controller that causes the whole system to run out of control. With linkage in series the system can tolerate the failure of the first order controller to switch OFF, but it shuts down if this controller fails to switch ON, as Fig. 3b shows. With linkage in parallel the position is reversed: a failure of the first order controller to switch OFF can be tolerated, but if he fails to switch ON, the second order controller is powerless to intervene.

Clearly, if a linkage in series is adopted, it is necessary to minimize the risk of the first order controller committing a failure of type 1 (Table 1). This can be achieved by designing the controller with a low p_1 (=probability of failure to switch ON when necessary). Alternatively, by coupling two (or more) controllers in parallel to act together as a first order controller, the probability of failure to switch ON is drastically reduced (Table 3) and, when this parallel linkage is connected to a second controller in series, the risk of a complete breakdown (Fig. 3b) is reduced.

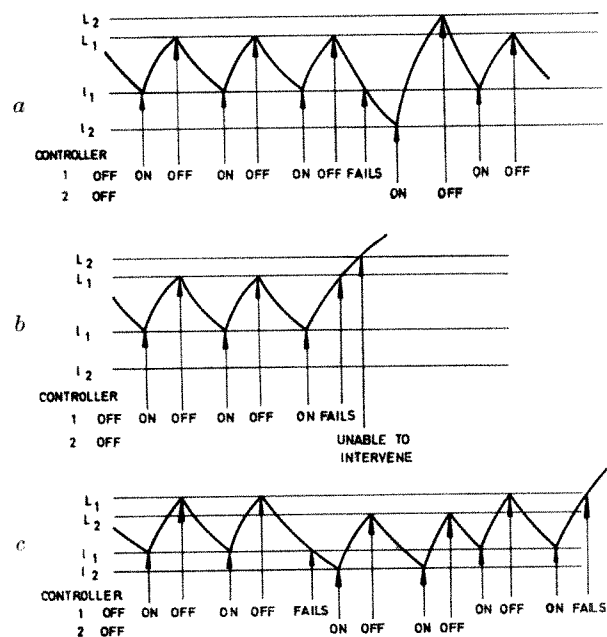


Fig. 4. Second order control in parallel.

Similarly, if a linkage in parallel is adopted between a first and a second order controller, it is desirable to avoid a situation such as shown in Fig. 4b, by replacing the single first order controller by two (or more) identical controls linked in series.

Conjoint Control

The operation of two non-identical thermostat controllers linked in conjoint control is shown in Fig. 5. In a $L_2 > L_1 > l_1 > l_2$ and the temperature fluctuates between l_1 and L_1 as long as the first order controller does not fail to act. When it does, the second order controller steps in and the temperature fluctuates then on the type of failure that the first order mechanism is subject to. If it fails to switch OFF, the control limits become effectively l_1 and L_2 ; and if it breaks down completely, the control limits are l_2 and L_2 . The failure of the whole system depends only on the failure of the second order controller,

and in this respect conjoint control differs fundamentally from the examples shown in Figs. 3 and 4.

Another mode of operation of first order and second order conjoint controllers is illustrated by two pairs of sieves through which beads of varying sizes are passed. (The beads are analogous to signals about the performance of the system.) The first order controller is represented by one pair of sieves: one sieve is for the lower limit, which removes beads that are too small; those that do not pass through this sieve are processed through another, which stands for the upper limit and does not allow too large beads to pass. If the second order controller is represented by another pair of sieves, one with holes smaller than the corresponding lower limit sieve of the first order and one with holes larger than the corresponding larger limit sieve, the second pair will not remove any beads if the first pair functions properly. But if the first order controller fails, or is removed, the second pair of sieves operate as if they were a first order controller, only the size of beads that are passed will fluctuate more widely than when the first original pair of sieves is used.

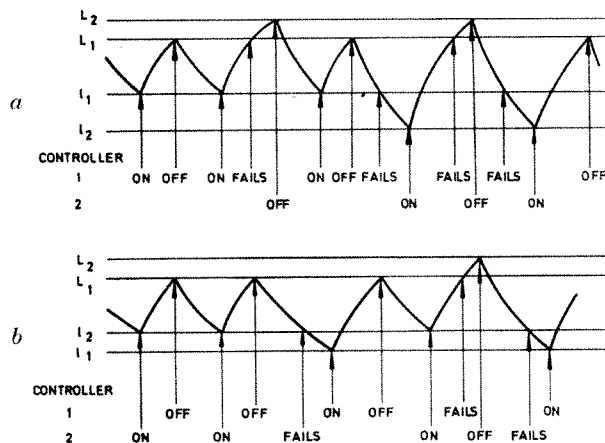


Fig. 5. Conjoint control.

Another type of conjoint control is illustrated in Fig. 5b where the control limits are such that $L_2 > L_1 > l_2 > l_1$. Here, in the absence of failure, controller 1 acts when the temperature reaches L_1 and controller 2 acts when it declines to l_2 . Thus the control function is shared by the two controllers: one guards against overheating, the other against overcooling. In this case, neither is a first order controller throughout, although each has a function attributable to a first order controller. Similarly, neither is a second order controller throughout, although each can act when the other fails. This mode of control can be called hybrid control.

Conjoint control can therefore take three forms: first order, when all controllers are essentially identical with respect to the stimuli to which they react; second order (or higher) when another controller takes over whenever the first order controller fails, and hybrid, when each controller performs functions of both first order and second order control.

The definitions of first and second order control and the examples cited earlier lead to several conclusions. (1) Two controllers acting on double action limits (Figs. 3, 4 and 5) are equal if $L_1 = L_2$ and $l_1 = l_2$ and the controllers are then

first order. (2) If a second order controller is identified in a system there must be at least one first order controller. If the latter breaks down, or is removed from the system, the second order controller takes over. (3) If two controllers are linked in series or in parallel the risk of certain failures in the control function is greatly reduced, compared with the single controller system, but the risk of a complete failure is increased (Table 3). If there is a second order controller this risk can be greatly diminished when the first order controller is replaced by two or more identical controllers linked in an appropriate way. (4) Hybrid control is a particular feature that can be built into conjoint control, but not into control in series or control in parallel. (5) Hybrid control can cause the system to be very tightly controlled and in administrative systems this can lead to acute frustration for controllers and the controlled.

Hierarchical Control

Hierarchy in an organization is often identified as an authority vested in one management level to intervene at another level and overrule decisions. As I have shown, even in pure series and parallel linkages of first order control each controller has some power over his fellow controllers, in the sense that he can, in certain circumstances, prevent the others from acting. It is useful therefore to define a hierarchical relationship between two controllers as allowing one controller to override another, but not vice versa. In other words, there is a distinction between the case when the overruling procedure is mutual for the two controllers (as in first order control) and when it is one sided.

In this respect second order control demonstrates some facets of hierarchical relationships, manifest by the fact that a second order controller need not act as frequently as a first order controller. The second order controller need not even monitor continuously the performance characteristics of the system or all the decisions of the first order controller, and arrangements can be made for certain cases only to be referred to the second order controller.

Second order control in series or in parallel gives the first order controller the power to lead the system to a state beyond the control of the second order controller. The power of intervention of the second order controller is therefore limited, as Figs. 3 and 4 show. On the other hand, the second order conjoint control allows the second order controller to intervene whenever the first order controller fails.

Another way in which hierarchical control can be designed is for control to be transferred from one controller to another, when certain symptoms in the operating characteristics of the system are unravelled. The procedure for general practitioners to refer patients to specialists or to hospitals is one such example. The decision to transfer the patient lies with the general practitioner; in other words the controller himself is responsible for identifying the circumstances in which he brings in another controller. In other cases the decision to transfer control may rest with a separate controller.

The types of linkage I have discussed are only three of many procedures that can be designed, but they illustrate well the way in which linkage between controllers can affect the relations between controllers and systems and interrelations between controllers.

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Scattered Arrangement of the Bacterial Ribosomal Cistrons

by

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In two bacterial species the tandem genes coding for 23S and 16S ribosomal RNA are widely scattered, by contrast with the situation in eukaryotes.

In both prokaryotes and eukaryotes, ribosomal cistrons occur as multiple genes. The only exception so far is the genome of *Mycoplasma*¹ which contains a single ribosomal cistron. In eukaryotes the ribosomal DNA complements are tightly clustered in the nucleolar organizer region(s) of the chromosome^{2,3}. The study of ribosomal DNA in *Xenopus laevis* and *Drosophila melanogaster* has shown that the 28S and 18S ribosomal DNA segments occur closely linked⁴⁻⁶ and that the nucleolar organizer DNA is made up from many linked repeats of such tandem genes⁴ together with spacer DNA which does not code for ribosomal RNA^{4,5,7,8}.

The bacterial 23S and 16S cistrons occur also as tandem cistrons⁹. Yankofsky and Spiegelman¹⁰ were first to propose a clustering of the ribosomal cistrons on the bacterial chromosome. Their hypothesis was borne out by observations that in both *Bacillus subtilis*¹¹ and *Escherichia coli*¹², ribosomal cistrons are located in two discrete regions of the genetic map. But less than 1 per cent of bacterial DNA is ribosomal DNA. The methods which have been used so far are unable to distinguish whether the several tandem pairs of 16S and 23S cistrons in each region are close together or scattered. Here we show that in at least two bacterial species the tandem pairs are quite widely scattered, by contrast with the situation in eukaryotes.

In the more commonly studied bacteria, ribosomal RNA has a GC content of some 53 per cent¹³ and from this one deduces that the ribosomal cistronic DNA in these bacteria bands in CsCl at a density of 1.710. In most of these bacteria the chromosomal DNA differs in overall GC content from that of ribosomal RNA. In these instances chromosomal DNA and ribosomal cistronic DNA have different buoyant densities. We shall show that this is the case, for example, for *Proteus mirabilis* (1.700), *Serratia marcescens* (1.718) and *Micrococcus lysodeikticus* (1.731 g cm⁻³) DNA. Bacterial ribosomal cistrons tend therefore to be embedded in genomic DNA of quite different buoyant density characteristics. This property can be used to study the degree of clustering on the chromosomal DNA.

Just under 0.4 per cent of the DNA extracted from an exponentially growing culture of *E. coli* K12 becomes hybridized with saturating amounts of ribosomal RNA¹⁴. In exponential growth (50 min generation time) the DNA content per genome is 1.4 times the genetic complexity¹⁵, so that this corresponds to $0.008 \times 1.4 \times 2,700 \times 10^6 = 30 \times 10^6$ daltons of (double stranded) DNA or ten copies of the ribosomal DNA cistrons. If we make the conservative assumption that all of the ribosomal DNA can be detected by hybridization and that the average position of the cistrons is halfway along the chromosome from the origin of replication, we can calculate that there are about seven copies of the ribosomal DNA cistrons per chromosome. If these are located in two regions in approximately equal numbers, each region contains three or four copies. Let us consider two extremes. First, all the ribosomal cistrons in each region are clustered together without intervening DNA. Because the amount of rRNA

corresponding to one tandem is about 3×10^6 daltons, three or four contiguous copies make up 9×10^6 – 12×10^6 daltons. Second, the three or four rDNA tandems in each region are widely separated by unrelated DNA sequences.

Whether we liberate pure ribosomal cistronic DNA fragments in these two cases will depend entirely on the size to which we fragment the chromosomal DNA. In the first case (Fig. 1a), fragmenting the DNA to, say, 6×10^6 dalton stretches will liberate nearly all rDNA free from other DNA, and only little ribosomal DNA will remain attached to other DNA. In the second case (Fig. 1b), 6×10^6 daltons are still enough to contain both ribosomal and intercalated DNA, in about equal proportions, and the overall buoyant density of the fragment would then be shifted towards that of the average genomic density position. To liberate pure ribosomal cistronic DNA in this case the DNA would have to be reduced in size to subcistronic levels.

To elucidate the nature of gene linkage of the bacterial ribosomal cistrons we banded bacterial DNA on CsCl gradients. We determined the position of the rDNA containing fragments on the gradient by challenging the fractions along the gradient with ³²P-labelled rRNA. In all gradients we included also a minute amount of *E. coli* ¹⁴C-DNA, for this bands at 1.710 as liberated bacterial rDNA should.

First we tested the proposition that pure bacterial cistronic DNA bands at 1.710 g cm⁻³. To eliminate any problems that might arise from gene linkage we sonicated the bacterial DNA to subcistronic levels (MW about 5×10^5 daltons) before centrifugation. Fig. 2a–c illustrates the outcome of the CsCl fractionation and hybridization experiment for *Proteus*, *Serratia* and *M. lysodeikticus* DNA. In all cases the ³²P-rRNA/DNA hybridization activity coincides more or less closely with that of the *E. coli* ¹⁴C-DNA marker, as expected. At the other extreme, when DNA has been prepared gently to avoid excessive fragmentation and banded on CsCl density gradients, it may be seen that the hybridization activity is close to that of the bacterial DNA as a whole (Fig. 3a–c). In these DNA preparations the ribosomal cistrons are still knitted in with other DNA.

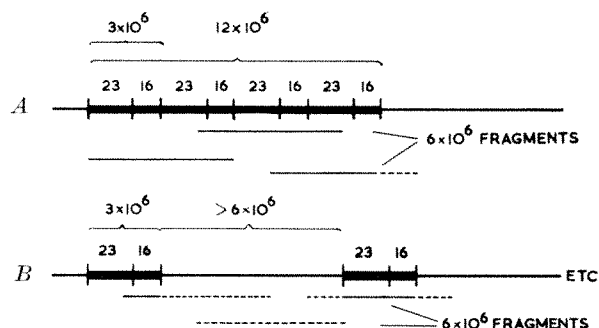


Fig. 1. Arrangement of the ribosomal 23S + 16S tandem genes.

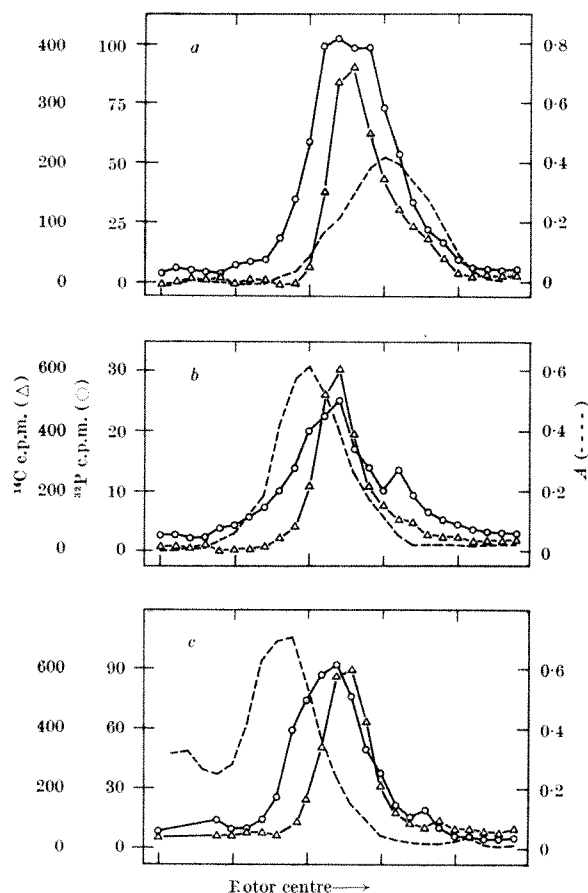


Fig. 2. Isopycnic centrifugation of liberated ribosomal cistrons. 100–150 μ g DNA was sonicated to a molecular weight of about 0.5×10^6 daltons and banded with 2 μ g *E. coli* ^{14}C -DNA in a CsCl density gradient (gradient B, ref. 4). Each gradient fraction was bound to membrane filters as described⁴ and challenged with 1–2 μ g/ml. *Proteus*, *Serratia* or *M. lysodeikticus* rRNA ($> 3 \times 10^4$ c.p.m./ μ g) in $6 \times \text{SSC}$ at 70°C for 2 h. The filters were treated with ribonuclease, washed by the batch method⁴ and radioactivity was counted. a, *Proteus* DNA; b, *Serratia* DNA; c, *M. lysodeikticus* DNA.

The banding of the rDNA with the remainder of the chromosomal DNA is not caused by an artefactual entrapment of ribosomal cistronic DNA. This can be seen in three ways: (1) the *E. coli* ^{14}C -DNA separates and attains its appropriate buoyant density, even when high molecular weight DNA is used; (2) reduction of the amount of DNA (5 μ g in Spinco angle rotor, No. 40 tube) yields an identical buoyant density for rDNA, and (3) when both *Xenopus* DNA (MW 25×10^6) and *Proteus* DNA (MW 7×10^6) are banded together (Fig. 4) the polycistronic *Xenopus* rDNA bands at 1.724 g cm^{-3} (ref. 4), while *Proteus* rDNA of intermediate size bands at intermediate buoyant density (1.705) between 1.712 (fully liberated) and 1.701 g cm^{-3} (fully integrated cistrons).

We have studied *Proteus* DNA at various stages of fragmentation. Fig. 5 summarizes these experiments. The average molecular weight of the *Proteus* DNA fragments is plotted against the average density of the rDNA-containing DNA segments. DNA which is smaller than 1.5×10^6 daltons contains DNA which bands like, or slightly heavier than, *E. coli* DNA (Fig. 2a). In DNA of 7×10^6 daltons the rDNA is obviously still contaminated by low density DNA (Figs. 4 and 5). Clearly the ribosomal tandem cistrons of *Proteus* are not clustered together, but are clearly spaced by other chromosomal DNA. Were this spacing relatively moderate, one might hope to arrive at a situation in which one could isolate serial repeats of intercalated and ribosomal tandem genes, as in rDNA of *Xenopus*⁴, and this would lead to a temporary levelling off and formation of a shoulder in the density shift. From

our results it seems that the first discernible levelling off occurs only in DNA with a molecular weight of the order of 30×10^6 . This, and the fact that in high molecular weight DNA the rDNA containing fragments band very close to the DNA as a whole, must mean that the length of the intercalated DNA is considerable. By balancing the density of the high density ribosomal cistrons against the low density chromosomal DNA we may estimate the approximate amount of intercalated DNA. If the intercalated DNA were to be made up from pure AT segments it would amount to 10×10^6 daltons. If it is of average base composition, the amount would be increased to 30×10^6 daltons.

The presence of intercalated DNA may also be detected in *Serratia* by hydroxyapatite fractionation. By this technique the compositions—but not the mass—of the intervening DNA can be determined. Because *Serratia* DNA is on average richer in GC than is *Serratia* rDNA it should be possible to choose a temperature of incubation at which ribosomal cistronic DNA is rendered single stranded while maintaining the spacer DNA in double strand form. The denatured DNA, if free from native DNA, may then be selectively eluted from the hydroxyapatite column and challenged with radioactive ribosomal RNA.

Fig. 6 shows thermal chromatograms of *Serratia* DNA at single stranded molecular weights of 0.7×10^6 , 1.5×10^6 and 6.6×10^6 daltons. 50 per cent of the ribosomal cistronic DNA is eluted at 92°C when the DNA is fragmented to sub-

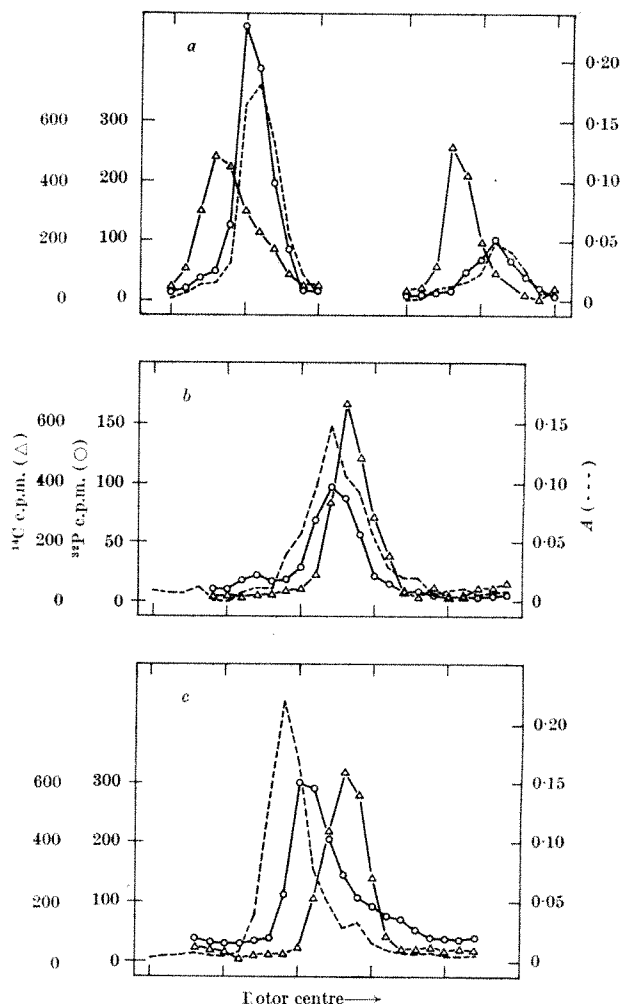


Fig. 3. Isopycnic centrifugation of integrated ribosomal cistrons. Conditions of centrifugation and hybridization as in Fig. 2, except that 50 μ g DNA was used for each gradient. a, 50 μ g (left) and 5 μ g (right) *Proteus* DNA about 70×10^6 ; b, *Serratia* DNA; c, *M. lysodeikticus* DNA.

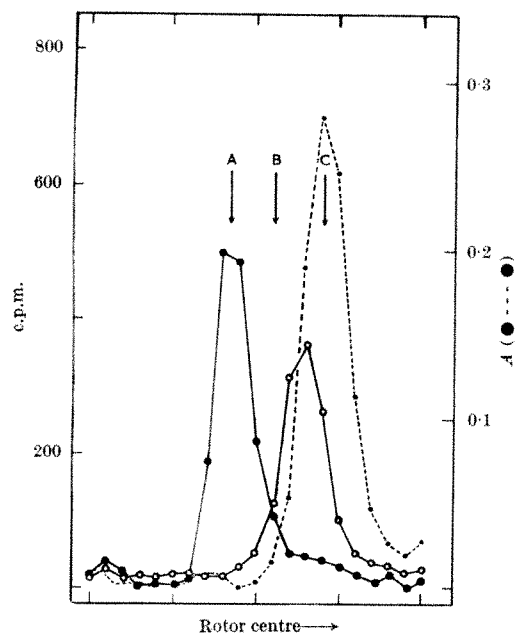


Fig. 4. Simultaneous banding of rDNA from *Xenopus laevis* and *Proteus mirabilis*. 25 μ g *Xenopus* DNA (MW 2×10^7) and 25 μ g *Proteus* (MW 7×10^6) were centrifuged together on gradient B* and challenged as described in Fig. 2, except that the hybridization mixture was incubated at 55°C and contained 50 per cent formamide, 6 \times SSC, 2 μ g/ml. *Xenopus* 3 H-rRNA (1.5×10^4 c.p.m./ μ g) (●—●) and 1 μ g/ml. *Proteus* 32 P-rRNA (○—○). A, *Xenopus* rDNA (1.724); B, free bacterial rDNA; C, *Proteus* and *Xenopus* DNA (1.698–1.700 g cm $^{-2}$).

cistronic levels: this is 4°–5° C below the temperature for the rest of the DNA. At 6.6×10^6 daltons, however, the elution profile of the ribosomal DNA sequences approximates that of total DNA, while at 1.5×10^6 , elution of the ribosomal cistronic DNA occurs at intermediate temperatures. At 6.6×10^6 daltons, melted ribosomal cistronic DNA is still physically linked to native DNA and thus retained on the column. Because the temperature necessary to elute rDNA in these conditions is close to that required to recover the main body of *Serratia* DNA it follows that the spacer DNA, at least in the vicinity of the ribosomal cistrons, must be of mean average base composition, that is, 58 per cent GC. Single stranded fragments of 1.5×10^6 daltons correspond exactly to a length of DNA sufficient to code for one 23S and 16S RNA. There is no reason to suppose that DNA breakage during shearing occurs only at the beginning or the end of the ribosomal tandem cistrons, so that 1.5×10^6 daltons stretches of DNA contain ribosomal cistronic DNA of varying purity and consequently with a chromatographic behaviour intermediate between the two extremes.

Xenopus laevis^{4,5,7,8} and *Proteus mirabilis* rDNAs resemble each other in that the ribosomal RNA sequences are interspersed with other DNA. In both cases the additional DNA is in large excess of the extra DNA necessary to account for the observed rRNA precursor. The precursor in *Xenopus* is 14 per cent larger than the sum of 18S and 28S RNA⁴, and in bacteria the relative difference is probably less^{16,17}. Here the resemblance ends. In the case of *Xenopus* the additional DNA is 1.2 times greater¹⁸ than or equal⁸ in quantity to the ribosomal cistronic DNA and has a very atypical base composition⁸. In the case of the bacteria the intercalated DNA seems to have average base composition, and is probably of the order of ten times greater in quantity than the rDNA.

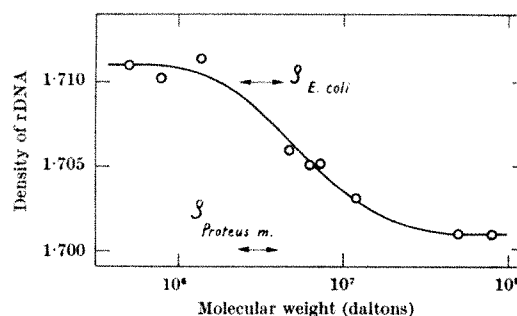


Fig. 5. Banding position of ribosomal DNA complements from *Proteus mirabilis* as a function of the molecular weight of the DNA. *Proteus* DNA was sheared or sonicated to various molecular weights, banded on CsCl gradients and challenged with 32 P-rRNA as described in Fig. 2. The mean density of the rDNA was determined for each preparation and plotted against the average molecular weight of the DNA preparation.

No function has hitherto been found for the bulk of the spacer DNA in *Xenopus*. In the case of bacterial rDNA the situation could be rather different. If we take three as a conservative estimate of the number of rDNA tandem cistrons located in the region of the *str* locus, then they span together with the intervening DNA a total DNA length of at least 30×10^6 daltons, corresponding to 1.1 per cent of the length of *E. coli* chromosomes or about 1.1 minute of map distance. In *E. coli* the region of the map around *str* contains a large number of known genetic loci¹⁹. It is therefore highly probable that some bacterial genes are located in the DNA regions which lie between the rDNA cistrons. The same may be true of *B. subtilis*. Smith *et al.*¹¹ have suggested that the rDNA cistrons are scattered in the region of *str*. Experiments which show that the DNA of the *str* gene and adjacent genes is rather resistant to thermal denaturation²⁰ suggest that these genes are closely associated with ribosomal cistronic DNA.

Our finding of the discontinuous arrangement of bacterial cistrons can also explain the difficulties encountered in attempts to isolate native ribosomal cistrons in pure form from bacteria^{21,22}. By contrast with *Xenopus*, where rDNA is a discrete DNA segment which can be separated with great ease from other DNA⁴, bacterial ribosomal DNA complements are necessarily more prone to be contaminated by chromosomal DNA. More sophisticated procedures for the isolation of native ribosomal cistrons from bacteria than those hitherto used are obviously required.

Fractionation and Hybridization of the DNA

The bacterial cells were lysed in 0.15 M NaCl–0.1 M EDTA–1 per cent sodium lauryl sulphate and deproteinized according

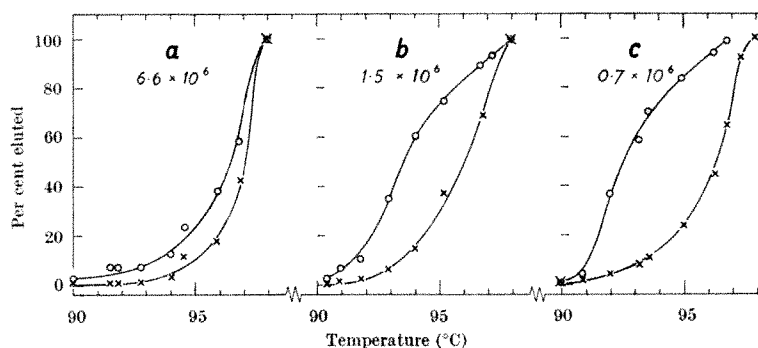


Fig. 6. Thermal chromatogram or hydroxyapatite columns of *Serratia* DNA at various molecular weights. 1 mg *Serratia* 14 C-DNA (630 c.p.m./ μ g) in 0.12 M phosphate buffer was bound to a 2×2 cm hydroxyapatite column²¹ at 70°C. The temperature was gradually raised and the denatured DNA eluted with 3×5 ml. 0.12 M phosphate buffer at 89–98°C in 1°C increments. 12 ml. of each fraction was denatured by addition of 1/10 volume of 1.44 N NaOH. 1/10 volume of 1 N H_3PO_4 together with 3/10 volume 20 \times SSC were added and the solution was passed through 'Millipore' filters. The immobilized DNA was reacted with 10 μ g/ml. *Serratia* 32 P-rRNA (8×10^4 c.p.m./ μ g) in 2 \times SSC at 65°C for 2.5 h. The filters were then treated with ribonuclease, washed and counted for 14 C and 32 P radioactivity²².

to Marmur²⁵. The DNA was further deproteinized by two consecutive treatments with pronase (0.2 mg/ml.) in SSC (0.15 M NaCl-0.015 M sodium citrate, pH 7.2) and by re-extraction with water saturated phenol. Traces of ribose oligonucleotides were removed by pelleting of the DNA overnight at 42,000 r.p.m., 20° C, in an MSE ultracentrifuge. The DNA was sheared by rapid stirring or by passage through a French Press at 6,000-20,000 pound/inch². The molecular weight of single stranded and native DNA was determined by the spill-over centrifugation technique as described in detail by Studier²⁶.

DNA was banded as described⁴. 5.2 g CsCl was mixed with 4 ml. 0.1×SSC containing sheared DNA together with DNA from *E. coli* labelled with ¹⁴C. The gradients were centrifuged to equilibrium in an MSE angle rotor²⁷ at 42,000 r.p.m., 25° C, for 36 h. Six-drop fractions were collected in 0.5 ml. 0.1×SSC. The absorbance at 260 nm was determined and each fraction of the gradient challenged with ³²P-labelled, chased rRNA as described earlier⁴ (see also legends to Figs. 2 and 4). The filters were washed according to the batch method⁴ and counted. Only machine background has been deducted from the measured radioactivity.

Note added in proof. Since submitting this article, we have learned from O. L. Miller (Oak Ridge) that when the *E. coli* genome is spread and examined in the electron microscope, he observed the scattered arrangement of the ribosomal DNA cistrons, in accordance with our conclusions for *Proteus* and *Serratia*.

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Determination of Diffusion Coefficients of Haemocyanin at Low Concentration by Intensity Fluctuation Spectroscopy of Scattered Laser Light

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Electronic analysis of optical signals, known as digital autocorrelation of clipped photon-counting fluctuations, has been used to measure diffusion coefficients of haemocyanin at concentrations ranging from 0.08 to 12.0 mg/ml. At 0.5 mg/ml., with laser power 30 mW, 1 per cent accuracy is achieved with a measurement time of only 30 s.

MEASUREMENTS of the diffusion coefficients of biological macromolecules in solution have sought chiefly to determine their molecular weights by applying the Svedberg equation, which relates these quantities to their sedimentation coefficient and partial specific volume. Until the early 1960s, this was the chief method for determining the molecular weights of proteins and, to a lesser extent, of polysaccharides¹⁻³. This led to the development of better methods for measuring diffusion coefficients^{4,5}. Accurate measurement of diffusion coefficients has always been difficult, however, because great care is needed in forming sharp, stable boundaries, because the optical components must be accurately aligned and because the measurements take a long time (from a few hours to days). This is a particular problem with materials of limited stability. Diffusion coefficients are frequently and readily determined by interference optical analysis of diffusing boundaries in a synthetic boundary cell in an ultracentrifuge, but their accuracy is seldom better than 5 per cent and, although stabilized, the diffusing boundary is inevitably disturbed in the rotating cell. Alternatively, the spreading of sedimenting boundaries may be used to determine diffusion coefficients, but the method is only accurate⁶ to

about 10 per cent, is complicated and is subject to limiting theoretical assumptions.

Because of these difficulties in measuring diffusion coefficients, sedimentation equilibrium methods are often preferred for molecular weight determinations, both because of their inherent accuracy and because of the great information that can be obtained from them. Most laboratories which have an ultracentrifuge, however, regularly submit their preparations of biological macromolecules to the quicker sedimentation velocity runs (if only for purely diagnostic purposes), and it would be a great help if an equally rapid and accurate method for measuring diffusion coefficients were also available. If diffusion coefficients could also be determined in a mixture of individual components which were identifiable as separate peaks in sedimentation-velocity runs, then estimates of their molecular weights could also be made without isolating them. We describe here a method for determining diffusion coefficients very accurately on small volumes at low concentrations by digital autocorrelation of scattered photons from a laser beam. Using this method, diffusion coefficients at various concentrations of haemocyanin from *Murex trunculus* have been determined.

Intensity Fluctuation Spectroscopy

Information about the distribution of relative velocities of pairs of particles taken from a set of randomly moving scatterers can be obtained from the spectrum of intensity fluctuations of scattered coherent radiation⁷. An excellent review is given by Atlas⁸. This technique was first used with coherent optical sources by Alpert *et al.*⁹ and by Ford and Benedek¹⁰.

A solution of macromolecules undergoing Brownian motion is just such a (Gaussian) source. The spectrum of scattered light can, in this case, be related to the diffusion coefficients which control the motion. Diffusion coefficients were first measured in this way in 1967 by Dubin, Lunacek and Benedek¹¹, although Cummins¹² made similar measurements in 1964 by a heterodyne technique. A third possibility has been demonstrated by Jakeman *et al.*¹³, who used statistical analysis of photon-counting distributions. The digital techniques used in this work can also be used directly to obtain the spectrum of scattered light. The information contained in the scattered light is, in this approach, retrieved from the train of points in time at which photodetection events take place at the detector. The probability of a photodetection is proportional to the intensity of the light, so that the fluctuations in intensity caused by beating together of the various Doppler-shifted frequencies are shown by bunching of the train at high intensities and thinning out at low intensities. The frequencies present in these intensity fluctuations are found by comparing the train with itself at a later time. The bunchings will match up one cycle later if a given frequency is present. This process of extracting spectral information is called digital autocorrelation.

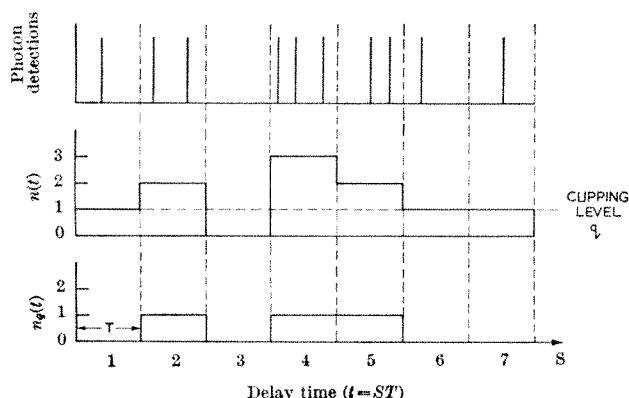


Fig. 1. A schematic indication of the clipped photon number $n_q(t)$. The sampling time T is the period of the shift clock of Fig. 2.

Foord *et al.*¹⁴ have found that direct autocorrelation of photon counting functions in real time is potentially the most accurate method. Although a full digital autocorrelator could be constructed, it would be expensive, and a simpler technique would involve very little loss in statistical accuracy. This is based on a method, originally formulated for use in the microwave region, which makes use of the fact that the zero crossings of a Gaussian signal contain most of the spectral information. These are the points in time at which the signal amplitude passes zero. The analogue of this microwave technique for use in optical spectroscopy has been given by Jakeman and Pike¹⁵. The principle is as follows. We introduce the quantities, for integral q , illustrated in Fig. 1

$$\begin{aligned} n_q(r, t) &= 1 \text{ if } n(r, t) > q \\ &= 0 \text{ if } n(r, t) \leq q \end{aligned} \quad (1)$$

where $n(r, t)$ is the number of photodetections over a small area A at r in a short time interval T at t . A single-

channel-clipped, normalized, autocorrelation function $g_q^{(2)}(\tau)$ may then be defined as follows

$$g_q^{(2)}(\tau) = \frac{\langle n_q(r, t) n_q(r, t + \tau) \rangle}{\langle n_q \rangle^2} \quad (2)$$

where the angle brackets denote a long-time average. For stationary Gaussian light, this may be expressed¹⁴ in terms of the optical correlation function $g^{(1)}(\tau)$ (the Fourier transform of the optical spectrum) as

$$g_q^{(2)}(\tau) = 1 + \frac{1+q}{1+\langle n \rangle} |g^{(1)}(\tau)|^2 \quad (3)$$

It is clear that a measurement of $g_q^{(1)}(\tau)$ would give the required spectral information. Moreover, it can be determined much less expensively than the full autocorrelation function because it involves multiplication only by zeros or ones, and this can be accomplished by simple binary circuits.

In the case of light scattered from the uncorrelated Brownian motion of monodisperse macromolecules, which are small compared with the wavelength, the optical correlation function is given by

$$g^{(1)}(\tau) = e^{-K^2 D_T \tau} \quad (4)$$

where D_T is the translational diffusion coefficient and K is the scattering vector. This equation is the three-dimensional Fourier transform of the expression given by Chandrasekhar¹⁶ (eq. 172) for the real-space correlation function. In work of high accuracy, equation (3) has to be corrected for the fact that A and T have finite values. Thus, for scattering from Brownian motion, we obtain finally

$$g_q^{(2)}(\tau) = 1 + f(A, T, \langle n \rangle, q) \frac{1+q}{1+\langle n \rangle} e^{-2K^2 D_T \tau} \quad (5)$$

The experimental determination of this quantity will be discussed in the next section.

Experimental Determination of $g_q^{(2)}(\tau)$

A diagram of the single-clipped digital autocorrelator is given in Fig. 2. Photons detected by the photomultiplier (I.T.T. FW130) are amplified and then standardized in a discriminator to give a train of pulses corresponding to the intensity fluctuations of the light incident on the detector. This pulse train is then limited in one channel by a set-reset flip-flop which gives an output in a sample time only when more than q pulses arrive. The clipped signal is moved sequentially down a shift register by a clock which determines the sample time. It is then cross-correlated with the original digital signal by making the elements of the shift register control the input gates to separate channels of a 1 MHz accumulator (a bank of counters). For the work described here, the light intensity was sufficiently low for $q=0$ to be used so that the arrival of one or more photons in a sample time entered 1 into the shift register. The distribution obtained clearly has the form $\langle n_q(0)n_q(ST) \rangle / \langle n \rangle^2$ which is the required $g_q^{(2)}(\tau)$ described above at $\tau=ST$; S is an integer corresponding to the channel of the store and T is the sample period. $N^2 \langle n_q(0)n_q(ST) \rangle$ corresponds to the contents of channel S of the store, $N \langle n \rangle$ to the total unclipped counts and $N \langle n_q \rangle$ to the total clipped counts where N is the number of samples. All of these quantities are recorded in counters. The contents of the store are therefore normalized against the product of the total counts and the total clipped counts to obtain $g_q^{(2)}(ST)$. By plotting $g_q^{(2)}(ST) - 1$ on a logarithmic scale against the time ST , equation (5) shows that a straight line should be obtained with slope $-2D_T K^2$. A typical result is shown in Fig. 3. D_T is usually corrected to the value of diffusion constant at 20°C in water, $D_{20, w}$.

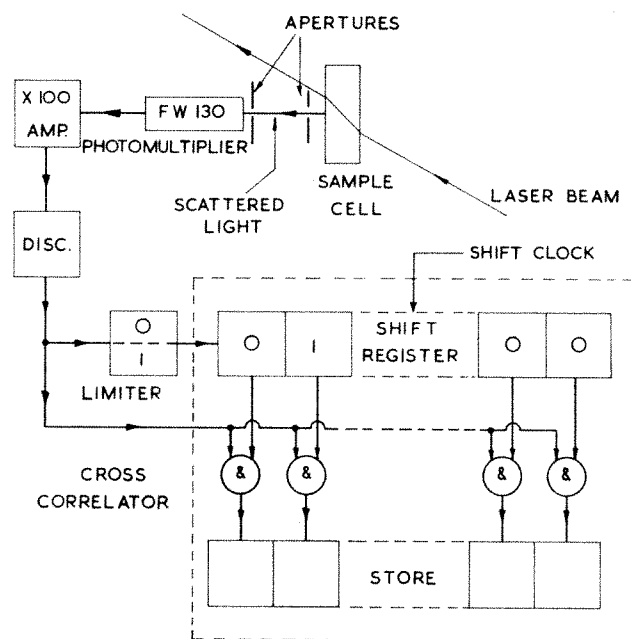


Fig. 2. A block diagram of the single-clipped digital autocorrelator.

The scattering vessels used for most of the work were spectrofluorimeter cells 2 cm \times 1 cm cross-section. Solutions and vessels were prepared by conventional techniques used in light-scattering studies. To reduce the effect of large impurities such as dust in the solution, the scattering was observed at an angle of 90°. The focused beam from a Spectra-Physics 125 He-Ne laser was scattered by the solution in the cells and a small solid angle of this light was defined by a two-aperture system. In this experiment, approximately 4 per cent of the coherence area and 10 per cent of the coherence time were used. This gives a value of 0.82 for $f(A, T)$ (unpublished results of E. Jakeman, C. J. Oliver and E. R. Pike). The system was aligned by a reference beam passing through both apertures to the photomultiplier and the light was reflected from the cell walls back along its own path. By ensuring that the light from the main laser beam incident on an adjacent face of the cell was also reflected normally, the two beams were known to be normal within the accuracy of the cell construction. It was necessary to achieve an

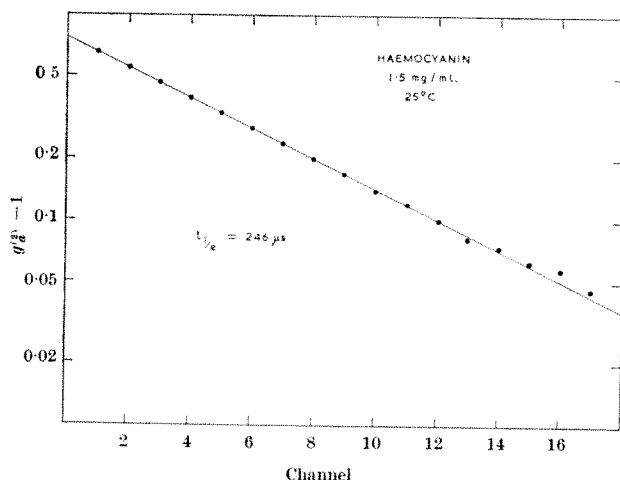


Fig. 3. A typical result for the autocorrelation function of haemocyanin. The concentration was 1.5 mg/ml. The diffusion coefficient $D_{20,w}$ was calculated to be $1.03 \pm 0.01 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ corresponding to an experimental time of 10 s.

angular accuracy of $\pm \frac{1}{2}^\circ$ for an overall accuracy of 1 per cent in D_T .

Applications of the Technique

One of the major disadvantages of earlier attempts¹¹ to determine diffusion coefficients of proteins in solution from line widths of a scattered laser beam was that high concentrations of the smaller globular proteins were required (scattering intensity is proportional to the square of molecular weight). We have therefore determined by the autocorrelation method the diffusion coefficients of two typical proteins in this lower molecular-weight range, bovine serum albumin (BSA; Armour Pharmaceutical Company) and lysozyme (BDH) at concentrations generally used in other hydrodynamic studies. Over the whole of the concentration range 0.5 to 20.0 mg/ml. the value of D_T for BSA in 0.1 M NaCl plus 0.01 M phosphate buffer (pH 6.0) at 20° was $5.70 (\pm 0.05) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ giving a value of 5.76 ± 0.05 for $D_{20,w}$. With lysozyme in 0.1 M NaCl plus 0.01 M phosphate buffer (pH 6.0), there was a slightly greater scatter of points in the concentration range 3 to 20 mg/ml., giving a larger error in the value of $D_{20,w}$ which was $10.6 (\pm 0.2) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$. Even with the most dilute solution of lysozyme (3 mg/ml.), an accuracy of ± 4 per cent was achieved in 2 h. Haemocyanin was obtained from specimens of the whelk *Murex trunculus* collected off the shores of Malta. Concentrations were determined from the absorption at 280 nm of solutions in 0.05 M borate buffer (pH 9.2) plus 0.01 M EDTA. At this pH the contribution of light scattering was greatly reduced¹⁷ and the extinction coefficient was $1.39 \text{ mg}^{-1} \text{ ml. cm}^{-1}$. The diffusion coefficient of *M. trunculus* haemocyanin has been measured over a wide range of concentrations in 0.1 M NaCl, 0.1 M acetate buffer (pH 5.7). In the concentration range 0.08 to 5.0 mg/ml., $D_{20,w}$ was $1.03 (\pm 0.01) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, rising to $1.08 (\pm 0.01) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ at 12 mg/ml. (Fig. 4). No deviation from a single Lorentzian distribution was detected in the range of angles 30° to 90°. The value obtained compares favourably with that of Lontie (unpublished results), namely $1.1 (\pm 0.05) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ at 11.5 mg/ml., measured on a Zeiss diffusion interferometer. Combination of our diffusion coefficient with the sedimentation coefficient for *M. trunculus* haemocyanin gives a molecular weight of $9.2 (\pm 0.1) \times 10^6$.

To show that the method is not limited by solvent, D_T for haemocyanin has been measured in 5 M guanidinium hydrochloride containing 0.5 M 2-mercaptoethanol. From the scattered intensity it was obvious that although the molecular weight was considerably reduced, it was still relatively high. Combination of D_T with the sedimentation coefficient gave a molecular weight of 210,000 ($\pm 20,000$), which suggests disruption into fortieths. This has been confirmed by conventional light scattering measurements. The only additional information required when working at high salt concentrations or in organic solvents is the refractive index of the medium.

It was possible to measure D_T for haemocyanin on only 15 $\mu\text{l.}$ of solution. The cell was a piece of capillary tubing of 1 mm internal radius and 0.5 cm long. At the concentration used, only 30 s of machine time were required to measure D_T to an accuracy of ± 1 per cent. Even at the lowest concentration used, 0.08 mg/ml., D_T could be determined to ± 1 per cent in three minutes. The statistical accuracy is roughly proportional to the inverse of the concentration and the square root of the total time of

Table 1. COMPUTED EXPERIMENTAL TIMES NEEDED AT DIFFERENT CONCENTRATIONS OF HAEMOCYANIN TO OBTAIN (a) 1 PER CENT AND (b) 0.1 PER CENT STATISTICAL ACCURACY IN D_T

Conc. mg/ml.	Mean number of photons per sample	(a) min	(b) min
0.08	0.017	3	300
0.13	0.028	1.5	150
0.47	0.1	0.5	50
1.5	0.3	0.17	17

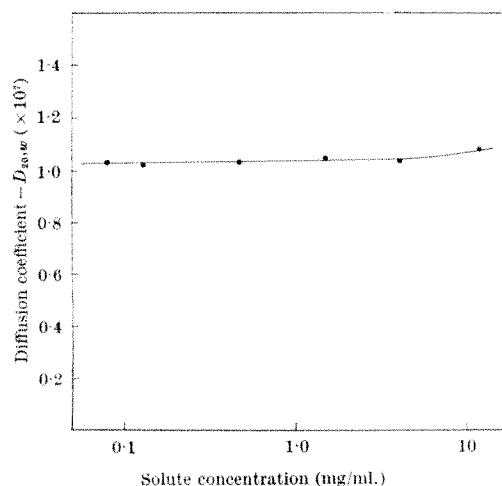


Fig. 4. The concentration dependence of $D_{20,w}$ for haemocyanin.

the experiment. Examples of computed experimental times required to attain statistical accuracies of 1 per cent and 0.1 per cent in D_T are given, for various concentrations, in Table 1. These agree with experimental results.

An interesting innovation made possible by this technique is the study of diffusion in mixtures of macromolecules. A preliminary study has been made on the apparently non-interacting mixture at pH 7 of bovine α -crystallin and haemocyanin in a weight concentration ratio of 10:1 to equalize the relative scattering powers. Although fitting the statistics to a sum of exponentials requires greater accuracy and longer experimental times (if a fit is forced to a single exponential, the result will approximate a weight average diffusion coefficient), the results were encouraging. Without any restraints at all in the fitting procedure, the D_T values were close to those observed for the separate components, although the relative proportions, as judged by the respective intensities, varied over a wider range. When the components interact, apart from the possible kinetic information available^{18,19}, the results should be useful both in detecting the presence of high molecular weight species and perhaps in estimating their size. This would help in the interpretation of other experiments on such systems. In a related

experiment, we have examined a solution of haemocyanin at an alkaline pH, where it is almost completely dissociated. The diffusion coefficients confirmed this and showed that the mixture contains mostly molecules of one twentieth and one half the original molecular weight.

The method reported here for measuring diffusion coefficients has several important advantages. There is no need to establish a concentration gradient, nor is stringent temperature control necessary to maintain such a gradient. The diffusion coefficient can be determined very accurately because the process is automatically a signal-averaging method. Furthermore, its measurement is relatively quick and the calculations are straightforward because of the digital output. The method is valid for any of the solvents used in macromolecular studies, is completely non-destructive, and only requires small volumes of solution. The concentrations needed are quite low and are similar to those needed for other hydrodynamic studies.

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Presence of EB Virus Nucleic Acid Homology in a "Virus-free" Line of Burkitt Tumour Cells

by

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The presence of virus in Burkitt lymphoma cells is hard to confirm, but small amounts of viral DNA have now been revealed by hybridization with DNA from cells of the Raji line of the tumour.

THE viral aetiology of Burkitt's lymphoma has been suggested ever since its first description^{1,2}. The discovery of a herpes-type virus (EB virus) by Epstein *et al.*³ in tissue culture cells derived from these tumours, as well as the presence of high antibody titres against this agent in Burkitt lymphoma patients^{4,5}, supported the original hypothesis. In recent years considerable immunological evidence has accumulated which points to an interaction between EB virus and Burkitt tumour cells⁶⁻¹¹, and the

relationship of EB virus to another lympho-proliferative disorder, infectious mononucleosis, has been demonstrated^{12,13}. Nevertheless, the role of EB virus in the aetiology of any human malignancy is far from being established.

Recently, it became feasible to isolate DNA from partially purified EB virus^{14,15}, making it possible to determine whether or not viral nucleic acids are present in tumour cells. In addition, one can now decide whether

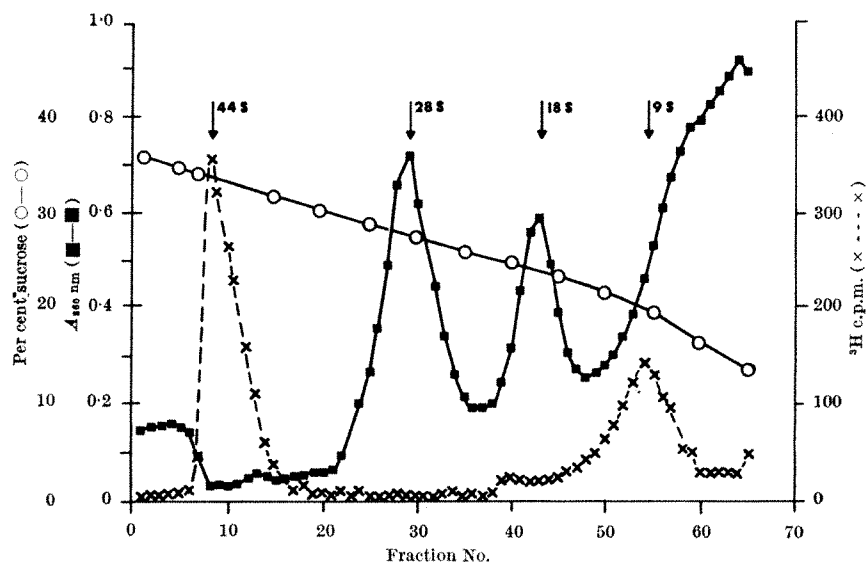


Fig. 1. Sucrose velocity sedimentation of EB virus ^3H -DNA, isolated from virus concentrates without deoxyribonuclease treatment before extraction. The virus concentrate (0.3 ml.) was layered on top of a sucrose gradient (20–35 per cent) in a buffer solution containing (per 100 ml.): 86.5 ml. reticulocyte standard buffer (RSB)²⁵, 10 ml. 0.1 M EDTA (pH 7.5), 2.5 ml. 4 M NaCl, and 1 ml. 5 per cent SDS. A solution consisting of 0.25 ml. 5 per cent SDS, 0.15 ml. 0.1 M EDTA, 0.15 ml. pronase P (Serva-Chemie, Heidelberg) (10 mg/ml.), 0.55 ml. RSB, and 0.1 ml. of non-labelled P3HR-1 RNA (about 400 μg), was added to the gradient. The tubes were incubated for 30 min at 37° C and then centrifuged in the 'SW 25-1' rotor of the Spinco preparative centrifuge for 18 h at 70,000g at 25° C. The fractions were collected from the bottom and examined for absorbance at 260 nm, for radioactivity, and for sucrose concentration. The sedimentation coefficient was determined according to Martin and Ames²⁶ by using 28S and 18S ribosomal RNA of P3HR-1 cells as markers.

Koch's first postulate, which requires the presence of the inducing agent in lesions of a disease, is true for the EB virus Burkitt tumour system.

We describe here a system which tests for the presence of EB virus DNA in tumour cells. The experiments were carried out with the assumption that EB genomes persist in every tumour cell like other well known DNA tumour viruses. To test this, the homology between the DNA from purified EB virus and nucleic acid derived from Burkitt tumour cells was studied. The results demonstrate that a "virus-free" line of Burkitt tumour cells contains approximately six EB virus genome equivalents per cell, as well as virus-specific messenger RNA.

Test System

A non-virus-producing line, Raji, isolated from a Burkitt lymphoma, was chosen. This line was established by Pulvertaft¹⁶. The cells were repeatedly reported to be EB virus negative by immunofluorescence and electron microscopy^{6,17,18}. Before use they were again tested for EB virus specific immunofluorescence and also subjected to virus-concentration procedures after prolonged labelling with ^3H -thymidine¹⁵. Both procedures failed to indicate the presence of EB virus. Raji cells, as well as all other cells used, were examined for mycoplasma contamination and found to be negative. Nucleic acids from P3HR-1 cells served as positive controls. These cells were derived from the P3J line of Burkitt tumour origin by cloning¹⁹. Approximately 10 per cent were repeatedly found to be EB virus positive by indirect immunofluorescence. Nucleic acid from Nil-2 cells, a line of Syrian hamster origin²⁰, served as a negative control. Nucleic acid from human KB cells was included in all tests. The maintenance of the various lines has been reported previously^{21,22}. Some experiments were carried out with DNA from cells of the HeLa line, the human leukaemia lines RPMI 64-10 (ref. 23) and SK-L1 (ref. 24), primary human leucocytes, and from *Micrococcus lysodeikticus*. Biopsy material obtained from a Burkitt lymphoma (kindly

supplied by Dr George Klein, Stockholm) was also examined.

The method for extraction of cellular DNA has been described²⁵. The technique of Warner *et al.*²⁶ was used for isolating total cellular RNA. The isolation and concentration of EB virus particles from P3HR-1 cells, as well as the isolation of viral DNA by equilibrium centrifugation in CsCl , have been described in detail¹⁵. Alternatively, DNA was extracted from virus concentrates by treating the preparations on top of sucrose gradients with sodium dodecyl sulphate (SDS) and pronase P. They were subjected to sedimentation as described in the legend to Fig. 1. DNA-DNA hybridization tests were performed according to Aloni *et al.*²⁷, with minor modifications²⁵. On filtration of 100 μg of radioactive heat-denatured cellular DNA through Schleicher and Schuell membrane filters, about 80 per cent of the DNA was retained by the filters. The procedure of Fujinaga and Green²⁸ was used for DNA-RNA hybridizations.

Compared with other common human DNA viruses (for example, herpes simplex, adenovirus, vaccinia), the yield of viral DNA per cell was very low (approximately 2 μg from 4×10^9 P3HR-1 cells). This was the limiting factor in the design of our experiments. The specific radioactivities of the various ^3H -labelled viral DNA preparations varied between 19,000 and 22,500 c.p.m./ μg DNA.

Hybridization of Viral DNA with Cellular DNA

^3H -labelled EB virus DNA, obtained from partially purified and deoxyribonuclease-treated preparations and isolated by equilibrium centrifugation in CsCl ¹⁵, was used for initial hybridization experiments. The results of four tests are combined in Table 1. DNA derived from Raji cells and Burkitt tumour biopsy material annealed about twice as many counts as human DNA of non-Burkitt origin. The high background annealing of human cellular DNA with EB virus DNA, however, indicated the presence of contaminating cellular DNA in the viral DNA preparation. Further hybridization tests were therefore performed with viral DNA obtained by sucrose velocity sedimentation as described in Fig. 1. Two peaks of ^3H -labelled material were identified (Fig. 1). Most of the radioactivity sedimented at 44S; a smaller fraction at approximately 9S. Treatment of the partially purified virus preparation with deoxyribonuclease reduced the 9S peak but did not eliminate it. The same 9S material was recovered from tissue culture medium after virus had been removed by centrifugation.

Table 1. HYBRIDIZATION EFFICIENCIES OF EB VIRUS ^3H -DNA WITH DNA DERIVED FROM HUMAN AND HAMSTER CELLS

Cellular DNA from	No. of experiments	No. of filters tested	Percentage of input c.p.m. hybridized (range)
<i>M. lysodeikticus</i>	1	1	0.22
Nil-2	4	4	0.19 (0.14–0.26)
KB	4	6	0.54 (0.44–0.68)
HeLa	1	1	0.33
Primary leucocytes	3	3	0.63 (0.50–0.85)
RPMI 64-10	2	2	0.66 (0.53–0.79)
SK-L1	2	2	0.50 (0.36–0.64)
Raji	4	8	1.19 (0.78–1.68)
Burkitt biopsy W. N.	2	2	1.53 (1.38–1.68)
P3HR-1	4	4	10.22 (8.95–13.02)

Cellular DNA (100 μg per filter) was annealed with 10,000 (two experiments), 20,000 (one experiment), or 25,000 (one experiment) c.p.m. of EB virus ^3H -DNA, isolated by CsCl equilibrium centrifugation.

Table 2. HYBRIDIZATION OF 44S EB VIRUS ^3H -DNA WITH DNA FROM HUMAN AND HAMSTER CELLS

Cellular DNA from	C.p.m. hybridized	Percentage of input c.p.m. hybridized
Nil-2	22	0.22
Nil-2, infected with herpes simplex virus	26	0.26
KB	26	0.26
Raji	106	1.06

Cellular DNA (100 $\mu\text{g}/\text{filter}$) was annealed with 10,000 c.p.m. of EB virus ^3H -DNA, isolated by sucrose velocity sedimentation.

The 44S DNA was collected, dialysed and concentrated by evaporation. It was then used for hybridization with Raji, KB and Nil-2 DNA. In addition, a membrane filter containing DNA from Nil-2 cells, infected with 1 p.f.u./cell of herpes simplex virus and extracted 24 h after infection, was included in the test (Table 2). EB virus DNA, isolated by velocity sedimentation, gave no increased background annealing with human cellular DNA as compared with DNA from hamster cells. Infection of Nil-2 cells with herpes simplex virus for 24 h (almost every cell revealed cytopathogenic changes at this time) did not increase their capacity to anneal with EB virus DNA. DNA from Raji cells, however, hybridized with approximately four times as many counts. Thus the viral DNA preparations were free of detectable contamination with cellular DNA; moreover, there is no significant cross-hybridization between DNA from EB and herpes simplex viruses.

The 44S DNA was used to study its saturation level with Raji DNA (Fig. 2). DNA from KB and Nil-2 cells bound between 40 and 50 counts at an input of 10,000 c.p.m. DNA from P3HR-1 cells annealed with approximately 13 per cent of the input while Raji DNA bound 216 c.p.m. of a comparable input. At higher inputs a plateau was reached between 250 and 350 c.p.m. Thus Raji DNA was saturated with EB DNA at inputs between 10,000 and 20,000 c.p.m. By subtracting the background of KB and hamster DNA, about 200 c.p.m. of EB virus DNA annealed specifically with Raji DNA. The specific activity of the labelled viral DNA used in this experiment was 22,500 c.p.m./ μg , so that the 80 μg of cellular DNA bound to the membrane included about 0.009 μg of non-labelled viral DNA. The DNA content of a diploid human cell (Raji cells, used in these experiments, contain 47 and

48 chromosomes) is approximately 6×10^{-12} g (ref. 31). The weight of EB virus DNA should be identical to that reported for herpes simplex DNA (1.2×10^{-16} g) (ref. 32), for both sediment at 44S (ref. 33). These data permit a calculation of the number of EB genome equivalents per Raji cell: on this basis, Raji cells would contain on average six EB virus DNA equivalents per cell.

Hybridization of Viral DNA with Cellular RNA

The presence of DNA sequences in Raji cells which are homologous to EB virus DNA raised the question whether or not they are transcribed. This was studied by annealing cellular RNA with non-labelled viral DNA. The isolation of unlabelled viral DNA was more difficult to achieve than the purification of radioactive EB virus nucleic acid. Because of the low concentration of viral material, most of the purification steps had to be done "blindly"; only low yields of viral DNA have therefore been obtained and no saturation or competition experiments have been carried out. The viral DNA was only purified by equilibrium centrifugation in CsCl .

Table 3. HYBRIDIZATION OF NON-LABELLED EB-VIRUS DNA WITH CELLULAR ^3H -RNA

RNA from	C.p.m. hybridized with EB virus DNA ($\mu\text{g}/\text{filter}$)	
	0.2	1.1
Nil-2	3	57
KB	9	82
Raji	42	191
P3HR-1	58	236

EB virus DNA (0.2 or 1.1 μg per membrane) was annealed with 400,000 c.p.m. of cellular ^3H -RNA. The specific activities (c.p.m./ μg) for RNA derived from Nil-2, KB, Raji, and P3HR-1 cells were 28,300, 47,400, 46,600 and 9,550, respectively. Each filter containing EB virus DNA was controlled by an additional membrane treated with corresponding amounts of purified adenovirus type 4 DNA and annealed with equal counts of cellular ^3H -RNA. The hybridized counts in the table represent the additional radioactivity annealed with EB virus DNA as compared with the corresponding membrane containing adenovirus DNA.

The results of two preliminary experiments are combined in Table 3, and show that EB virus DNA hybridizes with Raji RNA and RNA from P3HR-1 cells to a similar degree. This should be the result of the low specific activity of the P3HR-1 ^3H -RNA preparation used, which was approximately five-fold lower than that of the Raji cells. The hybridization of ^3H -RNA derived from KB cells was close to the background level of Nil-2 ^3H -RNA. It does therefore seem that Raji cells synthesize an EB virus specific RNA, although additional experiments are needed to characterize this RNA and determine the extent of its transcription.

Our demonstration of the persistence of EB virus DNA, or parts thereof, in tumour cells serves to indicate that the EB virus has features characteristic of known DNA tumour viruses, although the state of the viral nucleic acids within the cell, and their biological significance, remain to be clarified. The feasibility of demonstrating the presence of EB virus nucleic acid in "virus-free" cells should provide an additional tool for studying the role of this virus in the induction of human malignancies.

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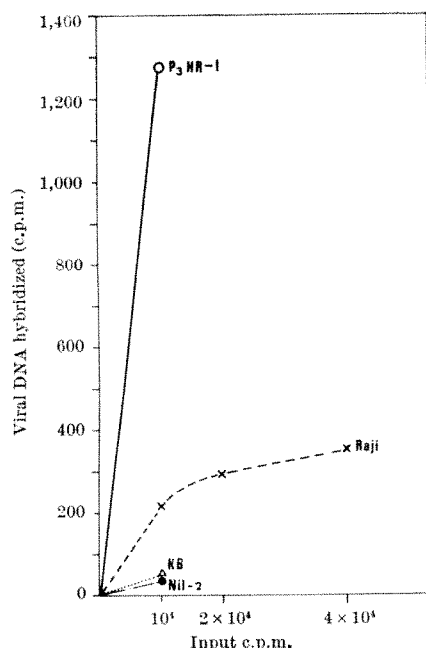


Fig. 2. Hybridization of Raji DNA (100 $\mu\text{g}/\text{filter}$) with 44S EB virus ^3H -DNA. DNA from Nil-2, KB and P3HR-1 cells was used as control.

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Genetic Analysis with Man-Mouse Somatic Cell Hybrids

Evidence of an active linkage between the human genes that control lactate dehydrogenase B and peptidase B is presented. It is also concluded that there is no link between the genes for lactate dehydrogenase A and lactate dehydrogenase B.

Linkage between Human Lactate Dehydrogenase B and Peptidase B Genes

By a genetic analysis of a series of enzyme markers in human-mouse somatic cell hybrids, we conclude that the human genes controlling lactate dehydrogenase B (LDH B) and peptidase B (Pep B) activities are linked.

Nine hybrid lines, each presumably originating from a different heterokaryon, were derived from a cross between 1-T, a subclone of a bromodeoxyuridine resistant, thymidine kinase deficient subline of the mouse cell line 3T3¹ (kindly provided by Dr H. Green) and peripheral white blood cells from a normal human female (for methods, see ref. 2). Eight of the nine hybrid lines are clones derived from a population of hybrid cells comprising several hundred original hybrid colonies. They were isolated about 30 days after fusion, and then grown as mass cultures. Line 3-31-Z resulted from a fusion of 1-T with white blood cells, which had been stored in liquid nitrogen³. It was chosen as an original hybrid colony. With some exceptions, all enzyme assays of the lines were carried out on the same batches of cells harvested at 75 to 120 days of culture after fusion. The cells were stored as washed pellets in Beckman tubes in liquid nitrogen and extracted by freeze-thawing in H₂O (50 μ l./2 \times 10⁶ cells) immediately before use.

Markers

The zymogram technique⁴⁻⁷ provides a particularly convenient and useful set of markers because it identifies primary gene products whose species of origin can usually be distinguished. Allelic variants are also, in general, codominantly expressed. The enzymes we have investigated are LDH A and LDH B (Fig. 1), Pep A, B, C and D (Fig. 2), supernatant NAD-dependent malate dehydrogenase (MDH), phosphoglucosmutase (human PGM₁ and PGM₂, see refs. 8 and 9), glucose-6-phosphate dehydrogenase (G6PD X-linked form), deaminase (ADA, ref. 10) the indophenol oxidase isozyme found in haemolysates (IPO, ref. 11) and phosphohexose isomerase (PHI, ref. 12).

The number of subunits in an autosomally inherited enzyme can be predicted from the number of intermediate bands in zymograms from heterozygotes for structural variants. In every case reported^{4-7,13} the number of intermediate bands, which represent interspecific heteropolymers in somatic cell hybrids displaying both human and mouse activity, corresponds to the number found in heterozygotes^{8-12,14-18}. Thus, we have found that MDH, IPO, Pep A and D and PHI can form one heteropolymeric band with intermediate mobility which is not observed, or occurs only as a trace, in extracts from cell

mixtures. Judging from the relative activities of the various bands, assembly of subunits usually seems to be random. PGM₁ and PGM₂, ADA and Pep B do not form heteropolymeric bands either in heterozygotes or in hybrids.

Table 1 gives the distribution of human enzyme activities in the nine hybrid lines. Where possible, a crude estimate of the relative amount of human, relative to mouse, enzymatic activity has been given. The contribution of the human form to the total activity is in general less than, and in no case appreciably greater than, that of the murine molecules. The simplest explanations for this observation are that whereas the heteroploid mouse parent line probably contains at least two copies of most genes, extensive human chromosome losses have eliminated, in most cases, at least one of a pair of homologues, and that within a hybrid cell line there may be considerable population heterogeneity with regard to human gene content. The second explanation is supported by a comparison between G6PD patterns from hybrids in which the X-chromosome is selectively maintained⁴ and patterns from other lines⁴, and by the correlation between the amount of human enzyme activity in a line and the

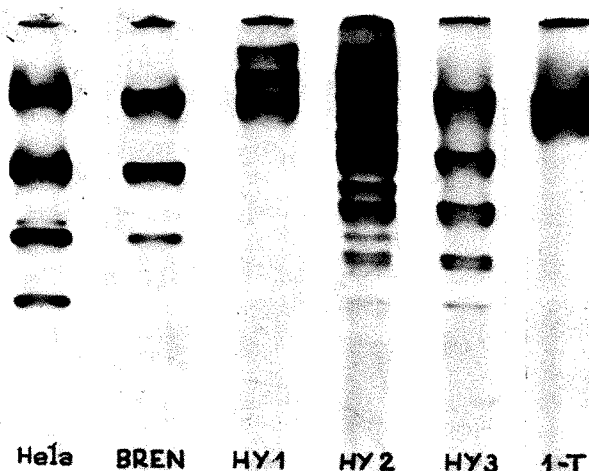


Fig. 1. LDH zymograms of hybrids and parental cells. Acrylamide gel electrophoresis was carried out⁴ on 5 μ l. of extract per sample. BREN fibroblasts are derived from the human donor in the cross; 1-T are cells in the mouse parent line. Hybrid 1 (HY1) shows the presence of human A subunits, hybrid 2 (HY2) has human A subunits and displays (compared with the mouse parent 1-T) an increase in LDH-B activity, probably because of the human LDH-B gene; hybrid 3 (HY3) shows only presumed human B activity.

Table 1. DISTRIBUTION OF HUMAN ENZYME ACTIVITIES IN HYBRID LINES

Line	LDH A	LDH B*	Pep B	Pep A	Pep C	Pep D	MDH	ADA	PGM ₁	PGM ₂	IPO	G6PD	PHI
3-31	-	±	±	-	-	-	-	-	-	-	-	-	-
4-11	-	+	+	+	-	(Pep D*) [‡]	-	-	±	+	+	-	+
4-12	+	+	+	+	+	(Pep D*) [‡]	+	+	+	+	+	-	+
4-21	+	+	+	+	-	-	-	-	±	±	+	-	±
4-22	-	-	-	-	-	-	-	-	+	+	-	-	-
4-31	-	+	+	-	-	-	-	-	+	+	-	-	-
4-42	+	-	-	±	-	-	+	+	-	-	+	-	-
4-43	+	+	+	+	-	-	-	-	-	-	-	-	+
4-45†	-	+	+	-	-	-	-	-	-	-	+	-	-
No. of positive lines	4	7	7	5	1	2	2	2	4	4	5	0	4

Where possible the amount of human compared with mouse enzyme activity is scored, as ±, + or ++.

* The basis for considering increased LDH B activity as attributable to human subunits is described in the text.

† The LDH pattern in line 4-45-Z at the time at which it was subclones indicated only a small amount of LDH B activity in excess of the 1-T parent.

‡ See legend to Fig. 2D.

Table 2. DISTRIBUTION OF HUMAN ENZYME ACTIVITIES AMONG CLONES DERIVED FROM VARIOUS HYBRID LINES

Line	No. of clones	LDH A	LDH B/Pep B	Pep A	Pep C	MDH	ADA	PGM ₁	PGM ₂	IPO	PHI
4-11-Z	1	-	+	+	-	-	-	+	+	+	+
	1	-	+	+	-	-	-	-	+	+	+
	1	-	+	+	-	-	-	+	+	+	+
	1	-	+	+	-	-	-	+	+	+	+
	4	-	+	+	-	-	-	-	+	+	+
	1	-	-	-	-	-	-	-	+	+	+
	1	-	-	-	-	-	-	-	-	-	-
4-12-Z	6	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+
	2	+	+	-	+	+	+	+	+	+	+
	2	+	+	-	-	+	+	+	+	+	+
	1	+	+	-	-	+	+	-	-	+	+
	1	-	+	-	-	+	+	-	-	+	+
4-31-Z	1	-	+	-	-	-	-	+	+	-	-
	1	-	+	-	-	-	-	-	+	-	-
	2	-	+	-	-	-	-	+	-	-	-
	3	-	+	-	-	-	-	+	-	-	-
	1	-	-	-	-	-	-	+	+	-	-
	1	-	-	-	-	-	-	+	-	-	-
4-42-Z	5	-	-	-	-	+	+	-	-	+	+
	7	-	-	-	-	+	-	-	-	+	+
4-45-Z	1	-	+	-	-	-	-	-	-	-	-
	7	-	+	-	-	-	-	-	-	-	-

Quantitative variations of activity are neglected.

proportion of clones derived from it, which contains this marker (compare Tables 1, 2 and 3).

Although no precise quantitative evaluation of the zymograms was made, it was clear that the levels of human and mouse enzyme activities were not closely correlated. Our previous data⁸ showed that high levels of human G6PD were conserved in hybrids derived from a cross with a hypoxanthine-guanine phosphoribosyl transferase deficient mouse line. None of the hybrids described here, derived from a cross between a thymidine kinase deficient cell line and white blood cells of a female with an A+B+G6PD phenotype, showed any detectable amounts of human G6PD.

In Table 1, it is assumed that the increased LDH B activity found in many hybrids is of human origin, although this cannot be demonstrated by electrophoresis. Patterns with increased B-subunit activity were previously only observed with human A subunit admixture⁸. The occurrence of this pattern among the hybrids described here (Fig. 1) argues against the possibility that some sort of regulatory effect related to the presence of human LDH A activity is responsible for an increase in the production of mouse B subunits.

Linkage Analysis

The distribution of human enzyme activities suggests close association between two pairs of human markers: the data from the lines indicate a linkage between LDH B and Pep B activities and between MDH and ADA activities.

These possibilities have been further investigated by a clonal analysis of the lines that carried these markers. Subclones were isolated at 55 to 75 days of culture after fusion. They were harvested about 40 to 50 days after isolation and assayed for the presence of human enzymes which could be detected in the line from which the clones were derived. The results of this analysis (Tables 2 and 3) confirm the association between LDH B and human Pep B activities found in the lines, and so indicate a linkage between the structural loci coding for LDH B and Pep B

in man. The possibility that the linkage involves regulatory rather than structural genes is, however, not ruled out. In particular it is conceivable that the structural Pep B locus is linked to a gene the presence of which leads to an increase in mouse LDH B activity in the hybrids and may also be necessary for human LDH B expression. Genetic regulation of LDH B activity has been demonstrated in mouse erythrocytes²⁰. The data given by Ruddle *et al.*²¹ in the accompanying paper make such an explanation extremely unlikely, however.

The complementary case of linkage between a gene regulating human Pep B activity and the structural LDH B locus would be expected to give rise to a situation in which human Pep B activity is only, but not always, there when human LDH B is present—a situation analogous to that found for MDH and ADA (see below). Strong selective forces may also lead to a complete coupling of the loss or maintenance of two human chromosomes carrying respectively the LDH B and Pep B genes.

The results obtained by Ruddle *et al.*²¹ from a cross involving a donor carrying an electrophoretic LDH B variant argue strongly against the involvement of a gene which regulates mouse LDH B activity. The clonal analysis of lines 4-12-Z and 4-42-Z argues against close linkage between the human structural genes coding for MDH and ADA. The data obtained from 4-12-Z are not very informative, as this line seems to be relatively uniform genetically and does not segregate for a number of the human markers. Whereas human MDH is present in

Table 3. DISTRIBUTION OF LDH-B AND PEP B ACTIVITY AMONG THE CLONAL OFFSPRING OF VARIOUS LINES

LDH B/Pep B* line	4-11-Z	4-12-Z	4-31-Z	4-45-Z	Total
+	7	13	6	0	26
±	1	0	1	1	3
-	2	0	2	7	11

* Complete linkage of human Pep B and increased LDH B activity, presumably because of the presence of human LDH B subunits, was observed. A rough quantitative evaluation of the zymograms suggests close correspondence of the activity levels of the two enzymes in the different cell populations. The frequency of the ++ clones suggests that their modal genotype contains one copy of the presumed LDH B/Pep B chromosome.

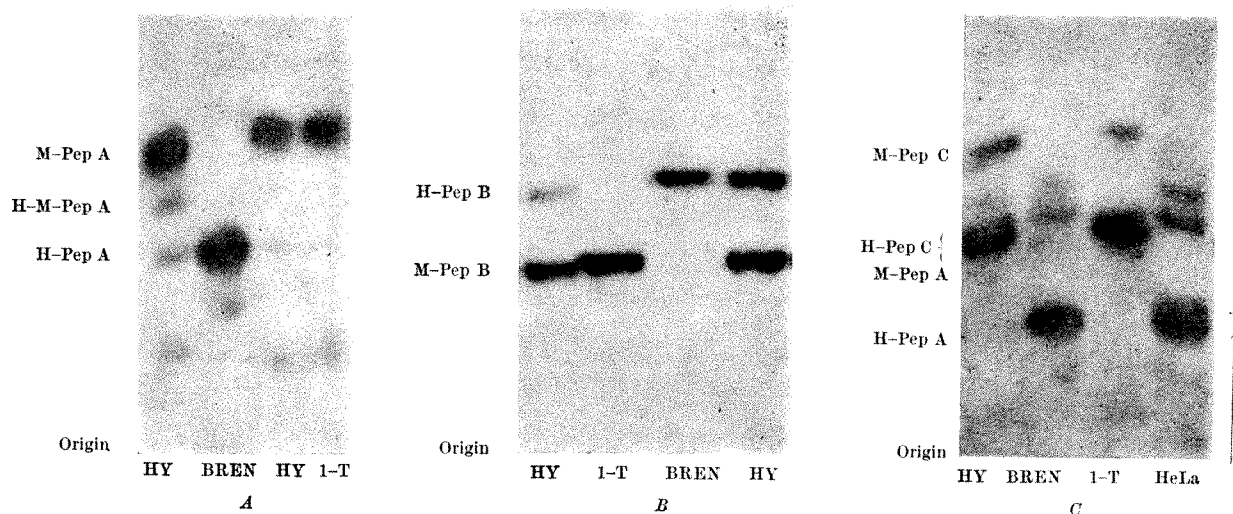
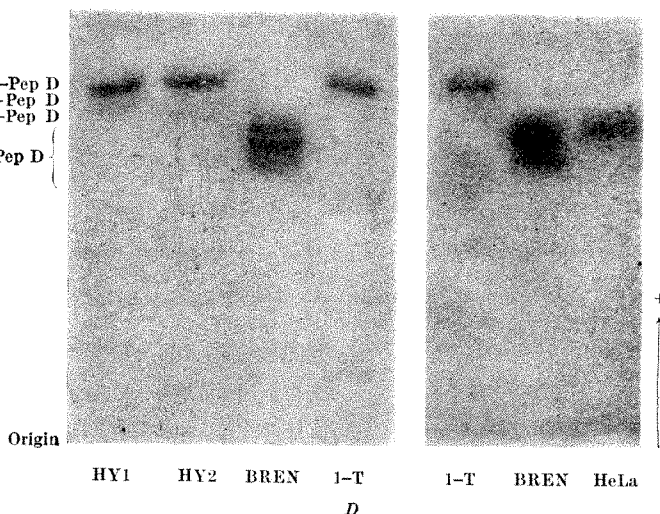


Fig. 2. Zymograms of peptidases of 1-T, the mouse parental line; BREN, fibroblasts derived from the human donor of the white blood cells for the cross and hybrid cell populations. 10 μ l. of extract was used for each sample. Starch gel electrophoresis and staining techniques were according to ref. 16. (A) Pep A: valyl-leucine was used as substrate. The slower band in the human fibroblast (BREN) is not found in haemolysates from the same person. We call mouse Pep A (M-Pep A) the only band observed in 1-T that forms hybrid molecules (H-M-Pep A) with human Pep A (H-Pep a) in the hybrids (HY). (B) Pep B; leucyl-glycyl-glycine was used as substrate. We designate as mouse Pep B (M-Pep B) the only band with substantial activity appearing with this substrate in mouse cell extracts. As in the case of the human Pep B activity, it is not seen with the substrates used to stain for Pep A, C and D. Hybrids show both a human and a mouse band. (C) Pep C: the substrate used, leucyl-alanine, can be hydrolysed by Pep C (H-Pep C) as well as Pep A (H-Pep A) in human haemolysates. In the mouse parent (1-T) a more anodic band appears in addition to the one designated as Pep A (M-Pep A). The patterns in haemolysates of the human white cell donor correspond to a normal Pep C 1 phenotype¹⁸. Fibroblast extracts of the same donor give a different pattern: in addition to the strongest band corresponding to the main Pep C zone in haemolysates, a faster migrating band, also found in HeLa cell extracts, is observed. These two bands were present in hybrids scored positive for human Pep C. In the fibroblast extracts a further slower moving band, not found in HeLa cell extracts, is seen. As it migrates at the same position as M-Pep A it could not be detected in hybrids. (D) Pep D: substrate was leucyl-proline. The human donor (BREN) displays a heterozygous phenotype, probably Pep D²-1 (ref. 17). In two hybrids, in addition to the only band hydrolysing this substrate in 1-T extracts, another band is found. The different positions of these bands suggest that only one human Pep D allele is present in each hybrid, namely Pep D¹ in HY1 and Pep D² in HY2 (see Table 1). These bands would represent molecular hybrids between the mouse enzyme and the respective human Pep D protein. Our interpretation of the peptidase patterns in mouse cells agrees with recent results obtained by Lewis and Treslove¹⁹.



all twelve clones derived from 4-42-Z, however, only five of them exhibit human ADA activity. Thus, wherever ADA is found, MDH is also present but the reverse is not necessarily true. If this association is confirmed, one of the following explanations seems most likely: the structural genes for MDH and ADA are linked in man but either (1) in line 4-42-Z an early translocation or deletion event produced a population component in which this linkage was disrupted and the human ADA gene was lost; or (2) in some 4-42-Z subclones human ADA activity is not expressed, because another human gene, not linked to MDH but necessary for ADA expression, is missing; (3) the complementary case to (2), namely linkage of such a "regulator" gene, but not the structural ADA locus, to the MDH gene would be expected to give the same result. Recent results²², which have indicated that in prokaryotes the level of species specificity of certain parts of the protein synthesizing machinery seems to be different for different genes, are of some interest in this context. Finally, an association between MDH and ADA could be the result of some form of interaction between the chromosomes carrying these markers which gives a selective advantage to hybrids that contain both elements.

Evolution of Hybrid Lines

We have previously suggested that hybrid lines quite rapidly reach a relatively stable equilibrium with respect to their content of human chromosomes⁶, and also that the evolution of the hybrids following cell fusion may be affected by quite strong selective pressures. Our new data further support these theories.

The LDH and IPO activities of the nine hybrid lines described here were assayed twice within a period of about 20 days, namely at about 80 and 100 days of culture after fusion. Two substantial changes indicating selective effects were observed. One line, 4-45-Z, showed a considerable increase in LDH B activity, whereas another line, 4-43-Z, lost its previously observed human IPO activity. (Cells for subclone isolation from 4-45-Z were plated out at the time at which the earlier assay was carried out.) In each case, the pattern observed in the first sampling did not correspond to that expected from random assembly of subunits and thus indicated population heterogeneity with respect to these genes.

The mean number of human enzyme activities detected per line was 4.6 (counting LDH B and Pep B as one) with

a variance of 10.5. This is not a Poisson distribution, and it indicates that the overall probability of retention of human chromosomes varies from line to line. There is, in addition, significant heterogeneity between the various enzymes with respect to the proportion of lines which have the respective activities. This suggests that some chromosomes may have a higher overall probability of being retained in the hybrids than others—a suggestion which has also been supported by preliminary karyotypic analysis (unpublished results of M. N.). There are also significant differences between the lines with respect to the proportion of clones which showed enzyme activity. The fate of a given chromosome may thus vary from line to line.

Clonal analysis of the various lines clearly demonstrates their individuality (see Table 2). Line 4-12-Z displays little or no segregation for five human enzyme activities which segregate frequently in other lines. For the other four enzymes present in 4-12-Z, however, considerable genetic variation apparently still existed at the time the subclones were isolated. Our previous results⁸ suggest that within a line stable segregating polymorphisms can exist—the fact that a gene segregates in subclones of a line does not necessarily mean that it will subsequently be lost from the population of cells that constitute the line.

If the very rapid early chromosome losses are caused by mechanisms different from the postulated selective pressures that seem to prevail later in the evolution of a line, then the curious fact that an enzyme, MDH, which was found in only two of the lines is so stably transmitted to their clonal offspring could be more than coincidental. Certain chromosomes that may initially be lost more rapidly than others may be expected to be found only in lines where they are maintained by relatively strong selective pressures.

Another important feature of the evolution of hybrid cell populations is the occurrence of associations between different human markers among subclones of one or more lines, which are not observed among the lines or among the clonal offspring of other lines. Data on PGM₂ and IPO which illustrate such pairwise patterns of association are shown in Table 4. There is no association among the lines, while the clones derived from 4-11-Z and 4-12-Z show some association. In the case of 4-12-Z, this simply reflects its tendency to retain more human chromosomes. Specific associations between pairs of chromosomes in clones from a given line could be caused by selective interactions between chromosomes. Marin (personal communication) has also suggested that they could arise from the elimination of associated sets of chromosomes during early mitoses following cell fusion. As already mentioned, such associations, whatever their origin, could lead in some cases to spurious apparent genetic linkages. Thus it is important to analyse clones derived from several lines when suspected linkages between unselected markers are to be tested. Further evidence concerning these possibilities emerges from karyotypic analysis of these hybrids (Razavi *et al.*, in preparation). This analysis is complicated by the relatively high level of karyotypic instability in heteroploid cell populations and in particular by the fact that bi-armed human-like chromosomes may be generated in the parent mouse cell line and in the hybrid lines derived from it. Thus, in some of the hybrid lines novel chromosomes were found that could be assigned neither to the normal human nor to the karyotype of the mouse parent line.

Our data seem to confirm the validity of an experimental design in which a relatively large number of independently isolated hybrid lines is screened as soon as possible after fusion for a panel of genetic markers. Significant associations can subsequently be tested by one or more subclonings from several lines. It is to be expected that other linkages will soon be established in this way. The next phase of this approach to somatic

Table 4. JOINT SEGREGATION OF PGM₂ AND IPO ACTIVITIES IN CLONES AND LINES

Clones from	PGM ₂ + IPO +	PGM ₂ + IPO -	PGM ₂ - IPO +	PGM ₂ - IPO -
4-11-Z	8	1	0	1
4-31-Z	0	3	0	6
4-12-Z	10	2	0	0
Lines	2	2	3	2

cell genetics will be to control recombination events and chromosome stability in somatic cell hybrids for the purpose of a linkage analysis within chromosomes.

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Linkage between Human Lactate Dehydrogenase A and B and Peptidase B

METHODS for establishing human gene linkage relationships, using somatic cell hybrids between man and mouse, have recently been developed^{1,2}. They are based on electrophoretic differences between homologous enzymes, on differences between chromosomes, and on the predominant diminution of only human chromosomes from the hybrids. The characterization of enzyme phenotypes in clonally derived cell populations containing different numbers and combinations of human chromosomes can be used to infer human gene/gene and gene/chromosome linkage relationships.

It has been suggested, on the basis of clonal differences in the activity of total LDH B, that the genes coding

for the A and B subunits of lactate dehydrogenase (LDH) are unlinked^{3,4}. Because the electrophoretic mobilities of the mouse and human B subunits are identical, the conclusion was only tentative, however, and other possibilities exist. For example, LDH A and LDH B structural genes may be linked, but other unlinked gene(s) may modulate the synthetic rate of mouse and human LDH B subunits. This suggestion is supported by genetic evidence for genes which control the expression of LDH B in mouse erythrocyte⁵.

We argued that the problem might be resolved using either a mouse or human structural variant of the LDH B subunit, and describe here a human LDH B variant, LDH B (var), and its segregation in clonal populations of mouse/human hybrids. This provides strong evidence for the absence of linkage between human LDH A and LDH B genes. Besides scoring the LDH phenotypes, we have ascertained the expressions of a total of fifteen different human enzyme phenotypes in a total of twenty-six clones and sixty-six subclones. These data suggest that the human genes for LDH B and peptidase B (Pep B) are linked, and this is supported by the accompanying report of Santachiara *et al.*⁶. Moreover, negative linkage of many other genes can be inferred.

Somatic Cell Hybrids and Clones

Peripheral leucocytes were collected from the proposita, J, and her mother, C. The leucocytes were separated from defibrinated whole blood by dextran sedimentation⁷, and then hybridized to mouse RAG cells using β -propionolactone-inactivated Sendai virus^{8,9}. The parental cells were mixed at a ratio of 1:1 at a total density of 4.0×10^6 per ml. in suspension with 1,000 h.a.u. of Sendai virus. The hybrids were selected in Dulbecco-Vogt modified Eagle's medium¹⁰ with 10 per cent newborn calf serum lacking γ -globulin and supplemented with hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), and thymidine (1.6×10^{-5} M) (HAT medium¹¹). The RAG parent is sensitive to this medium because it lacks hypoxanthine-guanine phosphoribosyl transferase (HG-PRT) activity⁸. Leucocytes do not proliferate *in vitro*. The hybrids proliferate in HAT because suppression of proliferation is recessive, and the HG-PRT deficiency in RAG is complemented by the unimpaired human HG-PRT gene. The selection system has been described in detail elsewhere^{2,8,9,11}. The mouse RAG cell line was derived from a renal adenocarcinoma of a BALB/c mouse. It is particularly appropriate for hybridization because of its relatively unmodified karyotype⁹.

Two independent series of hybrids were produced, and termed C and J after the respective human donors (Fig. 1). Mass cultures of C and J hybrids were recovered one month after hybridization. These possessed very weak activity for human LDH A and LDH B (var) phenotypes, indicating that only a few cells in the mass heterogeneous populations carried the LDH A and B (var) genes. The C and J populations were cloned to give the C and J series of independently derived clones. The LDH phenotypes of these clones were then examined to isolate hybrids possessing the LDH B (var) phenotype. Derivative subclones were produced from clones in the J series as shown in Fig. 1.

Linkage Relationships of LDH A and LDH B Genes

Previous studies have shown that lactate dehydrogenase is a tetramer¹². Two separate and distinct gene loci, termed A and B, code for subunit polypeptides which are distinct in their amino-acid composition¹³. The A and B loci are generally expressed in most somatic cell types, but in differing activity ratios¹⁴. The a and b subunits interact to give rise to five molecular forms of the tetrameric enzyme, namely, a_4 , a_3b , a_2b_2 , ab_3 and b_4 . These molecular forms possess different electrophoretic mobilities, and they correspond respectively to the LDH isozymes LDH-5, 4, 3, 2 and 1. The isozymes can be visualized by previously described electrophoretic procedures⁵.

Allelic variants at the LDH A and B loci have been reported in man¹⁵⁻¹⁸ and other mammals¹³. Analysis of the heritability of these variants indicates that the A and B loci are on autosomes. Because the variants are rare, no families which segregate variants for both A and B have been found, and the linkage of LDH A and B has not yet been determined in man. Genetically determined variants for LDH isozymes have been reported in fishes¹⁹. The number and complexity of LDH isozymes are greater in fishes than mammals, but A and B forms have been designated and homologized to the A and B forms in mammals¹⁹. Evidence that A and B variant forms segregate non-randomly²⁰⁻²² suggests that the loci are possibly linked. In mammals and birds an additional, unique form of LDH, LDH X, is expressed in testicular tissues and sperm²³. LDH X is coded by the LDH C locus. Recently, evidence has been presented that the LDH C and LDH B loci are very closely linked in pigeons²⁴. No evidence which demonstrates linkage relationships between LDH A and B in birds or mammals has been reported from genetic analysis *in vivo*.

LDH-5 homopolymers in mouse and man differ considerably in their electrophoretic mobilities and this provides good qualitative marker characteristics for mouse and human LDH A genes (Fig. 2: channels 8, 9 and 10). The LDH-1 homopolymers in mouse and man are not detectably different in their electrophoretic mobilities. The LDH B wild type mouse and human genes cannot therefore be distinguished in hybrid cells on a qualitative basis (Fig. 2: channels 8 and 10). Quantitative differences in the expression of mouse and human "b" subunits occurs, however, in the tissue cell lines used in the formation of the hybrids described here. Mouse LDH-1, 2 and 3 possess very reduced activities in the RAG cell line, and this is also true for other mouse lines such as A9 (Fig. 2: channel 9). Human "b" subunits are produced at a significantly higher rate in cell line WI-38 and other human cell lines such as KB, resulting in appreciable activity in LDH-1, 2 and 3 (Fig. 2: channel 8). Differences in activity of LDH-1 have been used to determine whether the human LDH B gene is present in hybrid cells between mouse and man³. It is possible, however, that regulatory phenomena could mimic such effects, and this makes it very desirable to use qualitative mutant forms of either the mouse or human LDH B gene for marker purposes.

An LDH B (var) allele was detected in the course of a

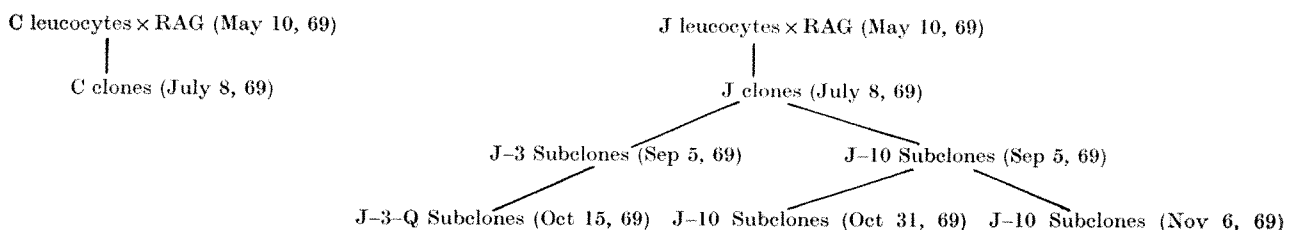


Fig. 1. Relationships of primary clones and derived subclones. Dates are given for the initiation of hybrids and the dates on which primary clones and derived subclones were picked.

Table 1. SEGREGATION OF LDH A AND LDH B (VAR) PHENOTYPES IN PRIMARY, INDEPENDENT CLONES OF C HYBRIDS

C	1	2	3	6	7	8	9	11	13	15	16	17	18	19	21
LDH A	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
LDH B (var)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

(-), No enzyme activity; (+), activity.

survey of newborn babies in the New Haven population²⁵. Only one variant form of LDH B was detected in 860 individuals sampled. The homopolymer LDH B (var) migrated slightly faster than wild type human LDH-2, and at approximately the same position as mouse LDH-2 (Fig. 3: channels 2 and 4). The family of the proposita was studied, and the inheritance of the variant allele determined. If one assumes codominance, the variant segregates as an autosomal gene, in agreement with previous family studies pertaining to LDH inheritance¹⁵⁻¹⁸. The variant was expressed as a heterozygote in all individuals who possessed it.

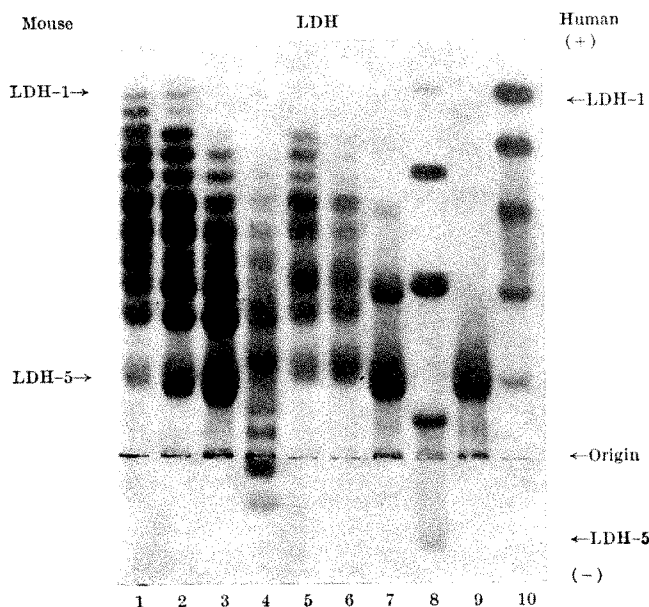


Fig. 2. LDH zymogram showing various mouse/man hybrid phenotypes. The channels were as follows: 1-7, mouse/man hybrid extracts; 8, human tissue culture extract (KB); 9, mouse tissue culture extract (A9); 10, mouse kidney extract. The human LDH phenotypes were scored as follows: (1) LDH A⁻/LDH B (var)⁺; (2) LDH A⁻/LDH B (var)⁺; (3) LDH A⁻/LDH B (var)⁺; (4) LDH A⁺/LDH B (var)⁺; (5) LDH A⁻/LDH B (var)⁺; (6) LDH A⁻/LDH B (var)⁺; (7) LDH A⁻/LDH B⁻. Gel electrophoresis was performed in a Tris-EDTA-borate gel at pH 8.6 (see ref. 5).

The patterns of LDH expression in the J and C clones provide strong evidence that LDH A and LDH B are not linked in man: clones C-21 and J-3, which both express the human LDH phenotype LDH A⁻/B (var)⁺, provide the critical evidence (Tables 1 and 2, Figs. 2 and 3). This configuration is more informative than the phenotype LDH A⁺/B⁻, because of the heterozygous nature of the human somatic cell parent, and the fact that the non-variant form of human LDH B is electrophoretically identical with mouse LDH B. The configuration LDH A⁺/B⁻ is less informative than LDH A⁻/B (var)⁺, because the human LDH B non-variant gene could in fact be present, but incorrectly scored because of its co-migration with mouse, or because of a regulatory phenomenon. The argument for non-linkage is greatly strengthened by the occurrence of phenotypic configurations in two completely independent series of clones derived from different donors (Tables 1 and 2). The independence of the observations

Table 2. SEGREGATION OF LDH A AND LDH B (VAR) PHENOTYPES IN PRIMARY, INDEPENDENT CLONES OF J HYBRIDS

J	1	2	3	4	5	6	7	8	9	10	11
LDH A	-	-	-	-	-	-	-	-	-	+	-
LDH B (var)	-	-	+	-	-	-	-	-	-	+	-

(-), No enzyme activity; (+), activity.

weakens counter arguments based on the possibility of chromosome fragmentation. The conclusion that LDH A and B are not linked is further supported by segregation of these markers in derivative secondary series of clones (Tables 3-7, and Figs. 2 and 3).

Table 3. SEGREGATION OF ENZYME PHENOTYPES IN SECONDARY CLONES OF THE J-3 CLONE

J: 3	A	D	E	G	I	J	L	M	N	O	P	Q	S	T
LDH A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDH B (var)	+	-	+	+	+	-	+	+	+	+	+	+	+	+
LDH B (act)	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Pep B	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ADA	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GOT	+	+	+	+	+	-	+	+	+	+	+	+	+	+
GPI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G6PD	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-), No enzyme activity; (+), activity.

It could also be argued that this interpretation might be incorrect because of the possible existence of genes which regulate the expression of LDH A. For example, human LDH A and B could indeed be linked, but a third unlinked gene might either specifically suppress or activate the expression of human LDH A. Two observations contradict this. First, the action of the repressor or activator would need to be species specific, for mouse LDH A is always expressed at uniform activity levels. Second, derivative subclones from J-3 (Table 3) and J-3-Q (Table 4) do not provide an example of a transition from human LDH A (-) to LDH A (+) as might be expected if an unlinked regulator gene were segregating. These arguments are not in themselves definitive, but they strengthen the conclusion that LDH A and B are not linked.

Linkage of Human LDH B and Pep B

In all our sixty-six derivative subclones there was a positive correlation between the expression of Pep B, and either the expression of human LDH B (var) or increased LDH B activity, which is presumed to indicate the presence of the human LDH B wild type allele (Tables 3-7). Appreciable activity of isozymes LDH-1 and 2 was used as a criterion for the presence of the non-variant human LDH B gene (Fig. 3: channels 3 and 5). The analysis of this presumed linkage relationship is unfortunately complicated by the apparent distribution of the LDH B non-variant allele to all of the subclone series. Nevertheless, clones which express LDH B (var) activity invariably also possess Pep B activity.

Other interpretations of the data are possible (Fig. 4). For example, the Pep B structural gene could be linked to a regulator gene which is required for the expression of mouse and human LDH B activity (hypothesis I;

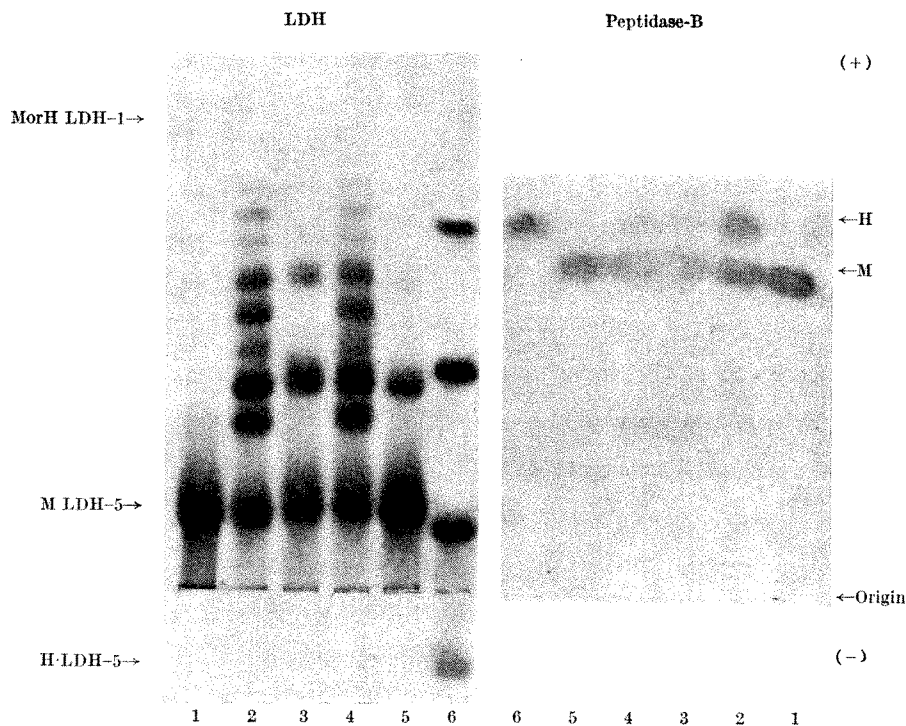


Fig. 3. Zymograms stained for LDH and Pep B activity. The slices were cut from the same gel. Channels having the same numbers represent identical samples. (1) Mouse control (A9); (2) mouse/man hybrid with an LDH A⁻/LDH B (var)⁺ human phenotype; (3) mouse/man hybrid with an LDH A⁻/LDH B (act)⁺ human phenotype; (4) mouse/man hybrid with an LDH A⁻/LDH B (var)⁺ human phenotype; (5) mouse/man hybrid with an LDH A⁻/LDH B-phenotype; and (6) human control (KB). The channels on the Pep B zymogram were similarly loaded and the human phenotypes were scored as follows: (1) mouse (control), (2) Pep B⁺, (3) Pep B⁺, (4) Pep B⁺, (5) Pep B⁺, and (6) human (control). The Pep B stain uses leucyl-glycyl-glycine as substrate²⁷.

Fig. 4; I). In that case, one would expect configurations of the type Pep B⁺/LDH B⁻ (Fig. 4; Ib). We have not observed such a combination in the J and C series of clones using activity levels of LDH B, or the presence of the LDH B (var), as criteria for the presence of human LDH B alleles. To be certain, our populations would have to be completely devoid of the wild type human LDH B allele.

One observation consistent with the linkage of Pep B and LDH B structural genes is that clones which possessed LDH B (var) activity frequently showed a concomitant increase in Pep B activity (Fig. 3), supporting a linkage involving the structural genes of Pep B and LDH B. Such a dosage relationship would not be predicted by hypothesis I (Fig. 4).

Table 4. SEGREGATION OF ENZYME PHENOTYPES IN SECONDARY CLONES OF THE J-3-Q CLONE

J-3-Q	1	2	3a	3b	4	7	8	9	10	11	12	13	14	15	16	19	20
LDH A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDH B (var)	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
LDH B (act)	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Pep B	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Pep A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GOT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GPI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G6PD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IPO	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
MOD	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MOR	±	-	-	-	±	-	-	-	-	±	±	-	-	-	-	±	-
PGM ₁	+	+	+	+	+	+	+	±	+	+	+	+	±	±	+	+	±

(-), No enzyme activity; (+), activity; (±), an equivocal result; elsewhere no determination was made.

Table 5. SEGREGATION OF ENZYME PHENOTYPES IN SECONDARY CLONES OF THE J-10 CLONE

J:	10	A	B	C	D	E	F	G	H	I	J	M	N	O	P	Q	S	T
LDH A	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+
LDH B (var)	+	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-
LDH B (act)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pep B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+
ADA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GOT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GPI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G6PD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IDH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IPO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MOD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MOR	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-), No enzyme activity; (+), activity; (±), an equivocal result; elsewhere no determination was made.

I LDH B regulator gene is linked to Pep B

Possible configuration	Expected phenotypes
(a) \uparrow Pep B \uparrow LDH B \downarrow reg	Pep B ⁺ /LDH B ⁺
(b) \uparrow Pep B \downarrow reg	Pep B ⁺ /LDH B ⁻ (species specificity) Pep B ⁺ /LDH B ⁺ (species non-specificity)
(c) \uparrow LDH B	Pep B ⁻ /LDH B ⁻

II Pep B regulator gene is linked to LDH B

Possible configuration	Expected phenotypes
(a) \uparrow LDH B \uparrow Pep B \downarrow reg	Pep B ⁺ /LDH B ⁺
(b) \uparrow LDH B \downarrow reg	Pep B ⁻ /LDH B ⁺
(c) \uparrow Pep B	Pep B ⁻ /LDH B ⁻

III Pep B and LDH B linkage units segregate concomitantly

(a) \uparrow LDH B \uparrow Pep B	LDH B ⁺ /Pep B ⁺
(b) 2 \uparrow LDH B \uparrow Pep B	LDH B ⁺⁺ /Pep B ⁺
(c) 2 \uparrow LDH B 2 \uparrow Pep B	LDH B ⁺⁺ /Pep B ⁺⁺
(d) \uparrow LDH B 2 \uparrow Pep B	LDH B ⁺ /Pep B ⁺⁺

Fig. 4. Hypotheses for the segregation of linked regulatory genes and non-random segregation of chromosomes as explanations for observed associations of Pep B and LDH B phenotypes. We assume that the presence of the regulator gene is required for the expression of the specified structural gene; that is, the regulation is positive. It is further assumed that only the non-variant form of human LDH B is used. Species specificity implies that the regulator affects the activity of genes within its own genome. According to hypothesis III, the numeral "2" signifies two chromosomes bearing the designated gene. (-) signifies no enzyme activity; (+) signifies activity.

Another possibility is that a regulator gene controlling Pep B activity is linked to the structural gene for LDH B (hypothesis II: see Fig. 4; II). This model predicts a segregant class which would express human LDH but not human Pep B (Fig. 4; IIb). In our observations, the LDH B (var) expression was always associated with the Pep B expression. This result therefore contradicts the alternative hypothesis II (Fig. 4).

A third alternative to the linkage of human Pep B and LDH B structural genes is that they reside on separate chromosomes which are either retained or lost from cells always concomitantly (hypothesis III: see Fig. 4; III). The large number of clones in our study and in that of Santachiara's *et al.*⁶ rules out a chance association of this type. It could be argued, however, that the simultaneous presence or absence of the chromosomes was co-adaptive in terms of affecting cellular viability, but this is made less acceptable by the dosage relationship between LDH B (var) and Pep B (Fig. 3). The co-adaptation argument is more difficult to accept if a 1:1 multiplicity relationship must prevail between the homologue carrying the human Pep B and LDH B genes, as is the case if the model is to agree with observations which indicate a dosage relationship.

In conclusion, the hybrid clone data most simply suggest that there is a linkage between the structural genes of LDH B and Pep B in man. More definitive evidence will come from a cytological search for association between the enzyme phenotypes and a particular chromosome(s). Chromosome studies on the J series clones are now being performed.

Linkage of Additional Isozyme Markers

We have also determined the expression of the following human isozymes in many of the subclones: Pep A, B, C^{26,27}; adenosine deaminase (ADA)²⁸; glutamate-oxaloacetate transaminase (GOT)^{29,30}; glucose phosphate isomerase (GPI)³¹; glucose-6-phosphate dehydrogenase

(G6PD)³²; isocitrate dehydrogenase (IDH)³³; indophenol oxidase (IPO)³⁴; malate:NADP oxidoreductase (decarboxylating enzyme) (MOD or NADP-MDH)^{35,36}; malate:NAD oxidoreductase (MOR or NAD-MDH) (ref. 37 and unpublished work of T. B. Shows, V. M. C. and F. H. R.); and phosphoglucomutase 1 (PGM₁)^{39,40}. There was no suggestion of positive linkage between any of the additional markers (Tables 3-6). Unfortunately, no positive statement can be made regarding negative linkages, because the data are based on segregation patterns in subclones, and not all clones were examined for all markers. Taking into account these limitations of subclone analysis², a summary of the linkage relationships is presented in Fig. 5.

Human G6PD activity was observed in every subclone. The X chromosome to which G6PD is linked would be expected to be retained in all instances because it also carries the gene for HG-PRT. If we assume that human HG-PRT is present in all clones, this would indicate that X chromosome rearrangements which would segregate HG-PRT and G6PD do not occur. The absence of such rearrangements in sixty-six subclones suggests either close linkage of the two genes or a low rate of X chromosome rearrangement or both.

Chromosome Constitution of the Hybrids

Preliminary analysis of the chromosome constitution of the hybrid clones has been performed. Human chromosomes were identified on the basis of arm length measurements, and their failure to anneal with mouse satellite DNA or its complementary RNA, using *in situ* cytological annealing (unpublished procedure of M. Pardue and J. Gall and ref. 40) (Figs. 5 and 6). The modal number

Table 6. SEGREGATION OF ENZYME PHENOTYPES IN SECONDARY CLONES OF THE J-10-M CLONE

J-10:	M	3	4b	6	7	9	10	11b
LDH A	+	+	+	+	+	+	+	+
LDH B (var)	-	-	-	-	-	-	-	-
LDH B (act)	+	-	+	+	-	+	+	+
Pep B	+	-	+	+	-	+	+	+
Pep A	+	+	+	+	+	+	+	+
Pep C		+	+	+	+		+	+
GOT	+	+	+	+	+	+	+	+
GPI	-	-	-	-	-	-	-	-
G6PD	+	+	+	+	+	+	+	+
IPO	+	-	-	±	-	+	-	+
MOD	+	±	-	-	±	+	-	+
MOR	+	-	-	-	-	-	±	-
PGM ₁		+	+	+	+	+	+	+

(-), No enzyme activity; (+), activity; (±), an equivocal result; elsewhere no determination was made.

Table 7. SEGREGATION OF ENZYME PHENOTYPES IN SECONDARY CLONES OF THE J-10-H CLONE

J-10: H	1	4	4a	5	6	7	9	10	11	12	13
LDH A	+	+	+	+	+	+	+	+	+	+	+
LDH B (var)	-	-	-	-	-	-	-	-	-	-	-
LDH B (act)	+	+	+	+	+	-	-	+	+	+	+
Pep B	+	+	+	+	+	-	-	+	+	+	+
Pep A	+	+	+	+	+	+	+	+	+	+	+
Pep C	+	+	+	+	+	+	+	+	+	+	+
GOT	+	+	+	+	+	+	+	+	+	+	+
GPI	-	-	-	-	-	-	-	-	-	-	-
G6PD	+	+	+	+	+	+	+	+	+	+	+
IPO	+	+	+	-	+	+	-	+	+	+	+
MOD	+	+	+	-	+	+	-	+	+	+	+
MOR	-	-	-	-	-	-	-	-	-	-	-
PGM ₁		+	+	+	+	+	+	+	+	+	+

(-), No enzyme activity; (+), activity; (±), an equivocal result; elsewhere no determination was made.

ADA	ADA	GOT	GPI	G6PD	IDH	IPO	LDH A	LDH B	MOD	MOR	PEP A	PEP B	PEP C	PGM ₁
GOT	-3													
GPI		-4												
G6PD	-3	-1	-5											
IDH		-1		-1										
IPO	-2	-2	-4	-2	-1									
LDH A	-1	-2	-3	-3	-1	-3								
LDH B	-3	-3	-5	-3	-1	-3	-6							
MOD	-1	-3	-4	-3	-1	-2	-2	-3						
MOR	-1	-4	-1	-5	-1	-4	-3	-5	-3					
PEP A	-1		-3			-2	-1	-3	-3	-3				
PEP B	-3	-3	-5	-3	-1	-3	-3		-3	-5	-2			
PEP C			-3			-2		-2	-2	-2		-1		
PGM ₁	-1	-2	-1		-3		-3	-2	-2	-1	-2			

Fig. 5. Phenotype association in two independent and seven derivative families of clones. Summary of segregation data shown in primary clones C and J, and secondary clones J-3, J-3-Q, J-10, J-10-M, J-10-H. Negative associations between pairs of phenotypes are measured in terms of numbers of clone series (not individual clones within a series) which show negative correlations. This was done because clones within a series may not represent independent segregation events. Zero scores indicate possible positive linkages. Many of the zero scores such as between PGM₁ and IDH are probably not significant because of absence of segregation and/or paucity of phenotype testing.

of human chromosome numbers varies between ten and sixteen in most clones. All classes of chromosomes are present, and identification of human *D* and *G* chromosomes can be obtained by the *in situ* annealing technique. Because the subclone series differ with respect to the presence or absence of LDH A, it was possible to confirm our previous linkage assignment of LDH A to a *C* group chromosome (unpublished results of C. Boone and F. H. R.). In most clones, it was possible to identify a human *C* group chromosome with arm length measurements which were consistent with those of the human *X* chromosome.

In conclusion, we agree with Santachiara *et al.*⁶ that the strategy for future linkage studies will involve the isolation of large numbers of primary, independently derived hybrid clones which can be analysed for a large number of unit markers such as antigens, isozymes, as well as chromosomes. In instances where ambiguities exist, subcloning can be used to resolve them. We believe the method will contribute powerfully to our knowledge of human gene linkage relationships.

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We learn that Dr Westerveld and Dr Bootsma of Rijswijk, The Netherlands, using human-Chinese hamster somatic cell hybrids, have recently obtained data (unpub-

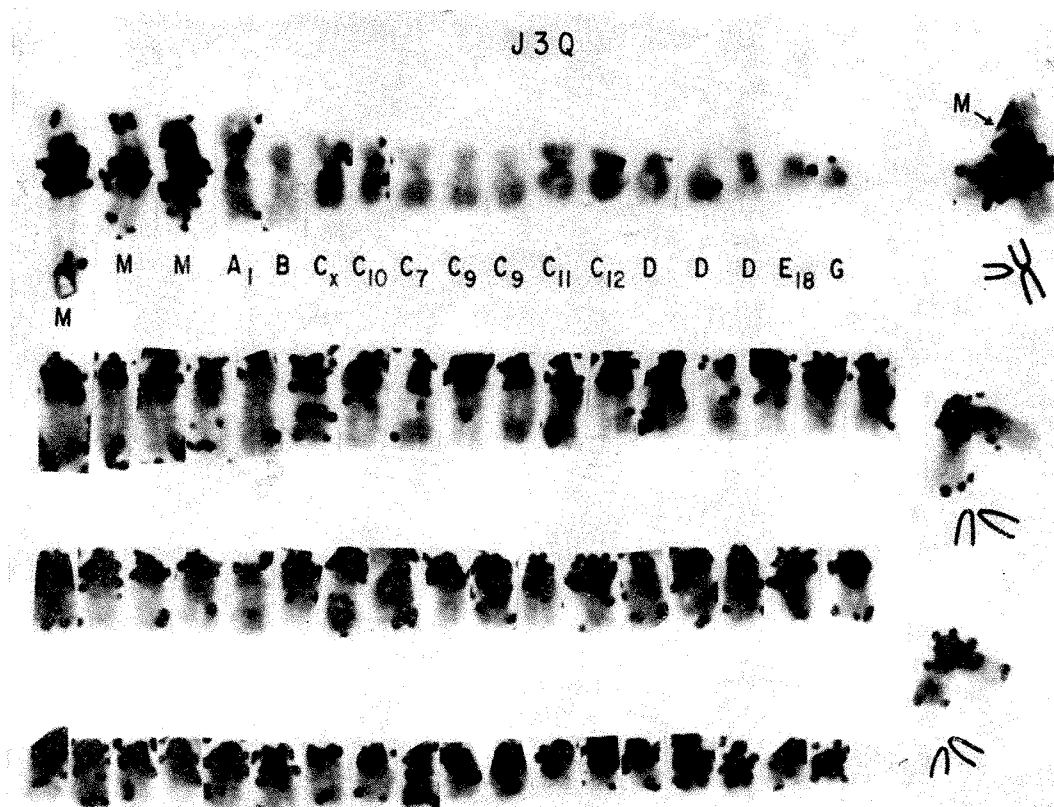


Fig. 6. Verification of human chromosome identity by means of nucleic acid annealing. Mouse bi-arm chromosomes are identified by the symbol "M"; the various types of human chromosomes by their group designations as stipulated by the London Conference¹³. Determination of human chromosome identity was made on the basis of absolute length and centromere position. Preparations were made by the air-dry method of Moorhead *et al.*¹³. The fixed, air-dried metaphase preparations were incubated with isotopically labelled RNA complementary to mouse satellite DNA. This material specifically anneals to mouse satellite DNA which is located in the centromere regions of the intact chromosomes. In autoradiograms, the chromosomes of mouse origin are isotopically labelled over their centromeres. Human chromosomes are unlabelled. The method especially permits the more certain identification of human *D* and *G* chromosomes.

lished) which confirm our observation on the independent segregation of human LDH A and LDH B loci.

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Evolution of Lorises and Lemurs

by

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Field studies in Madagascar and Gaboon have shown that there are many ecological and behavioural similarities between Cheirogaleinae (mouse and dwarf lemurs) and Galaginae (bush babies), which may reflect the retention of ancestral primate features. If this is so, the relationships of lemurs and lorises in previous primate classifications will need to be revised.

CLASSIFICATIONS of the order Primates attract great attention because of their special relevance to human evolution. Many different schemes have been proposed; but most work has been concentrated on relationships within major divisions and on the ranking of divisions within the Primates. The divisions themselves have remained largely unquestioned over the past 50 years, following widespread acceptance that there are six "natural groups" of living Primates: (i) Malagasy lemurs (lemur group); (ii) bush-babies, pottos and lorises (loris group); (iii) tarsiers; (iv) New World monkeys; (v) Old World monkeys; and (vi) apes and man.

Previous Classifications

The chief points of contention have been: inclusion of the tree shrews (Tupaiaidae) with the Primates¹⁻³; ranking of the Tarsiiformes (tarsier group) with either the prosimians (lemur group + loris group)¹ or the simians (monkeys, apes and man)^{4,5}; the inclusion of various fossil groups⁶⁻⁸; and the fine details of hominid (human) evolution. All authors seem to agree on a basic division between prosimians and simians and on the existence of six groups. Indeed, the tendency is to relate various fossils to one or other of these groups, without considering the evolution of the Primates as a whole. In particular,

fossils and living forms of dubious Primate status (namely the tree shrews) are typically placed with the lemurs, widely regarded as the most primitive living Primates.

Modern classifications, besides providing a useful reference system, should be consistent with supposed evolutionary relationships. The assumption that the lemur group and the loris group represent two distinct categories (variously ranked as families⁹, superfamilies, infraorders¹, or suborders^{4,10}) is clearly linked to the supposition that each of the two groups contains a range of forms derived from a distinct ancestral stock. This fact is not always clear. Confusion arises, first, because of the lack of clear guidelines in considering primate evolution³ and, second, because the published classifications are seldom accompanied by a hypothetical phylogenetic tree. Many authors hesitate to append an evolutionary tree, because much speculation is involved; but, on the other hand, the value of a classification is limited without some outline of the way a particular group has supposedly evolved.

The "ancestral Primate stock"³ is generally thought (or implied) to be located in the Upper Cretaceous and/or Palaeocene, while the existing and subfossil Malagasy lemurs are thought to have been derived from a single stock isolated on Madagascar in early Eocene times¹¹, primarily as a result of continental drift. Thus a definite

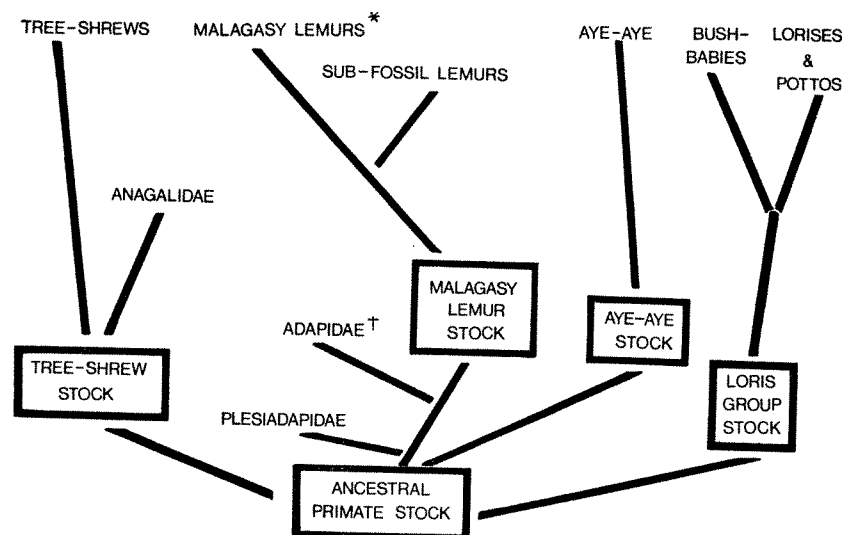


Fig. 1. One of several possible evolutionary trees for the lemurs and lorises derivable from the classification used by Simpson and Le Gros Clark. (Ancestral stocks indicate implied major subdivisions.) Note that the tree shrews, lemurs and aye-aye are placed in one infraorder (Lemuriformes), separate from the loris group infraorder (Lorisiformes).

* "Malagasy lemurs" refers to living Madagascar prosimians, excluding the aye-aye.

† The family Adapidae is frequently split into two groups: the family Adapidae (European forms) and the family Notharctidae (American forms).

Malagasy lemur stock is generally assumed to have existed in the Eocene, perhaps persisting for some time. No such clear-cut date has been suggested for the occurrence of a loris group stock, but Walker would apparently locate this in the Miocene¹². In any case, according to much current reasoning, the distinct stock giving rise to all the Malagasy lemurs (living and subfossil) must logically have been separate from the line leading to the loris group since the early Eocene.

Position of the Tree Shrews

This picture of prosimian evolution is made more complex by inclusion of the tree shrews in the Primates^{1,2}, because Simpson and Le Gros Clark both rank these mammals together with the Malagasy lemurs in the infraorder Lemuriformes, distinguishing these sharply from the loris group (infraorder Lorisiformes). If one follows this classification without the aid of a phylogenetic tree, several interpretations are possible; different patterns of relationship are consistent with the classification. One possible corollary is that tree shrews are more closely related to lemurs than the latter are to lorises, though neither author actually advocates this conclusion. An outline phylogenetic tree closer to the published opinions of these authors is given in Fig. 1, which shows that some features of the classification may be inconsistent with the probable evolution of prosimians. For example, Simpson places *Plesiadapis* as a Palaeocene relative of Malagasy lemurs in the superfamily Lemuroidea, apparently implying that many typical lemur characters (opposable digits, post-orbital bar, tooth-comb, and the like) had not been developed in the ancestral lemur/loris stock. Yet there can surely be no doubt that lemurs and lorises are far more closely related to one another than either group is to *Plesiadapis* or the tree shrews. In fact, much recent evidence shows that the tree shrews probably resemble Primates only in the retention of ancestral placental mammal characters and in the convergent development of adaptations found in many arboreal mammals. Because Simpson included *Plesiadapis* in the Primates at least partly on the grounds of comparison with (some) living tree shrews and (some) living Primates¹³, this allocation should also be reviewed.

Are the Distinctions between Lemurs and Lorises Valid?

One must now ask whether the traditional distinction between lemurs and lorises is really valid; that is, whether Fig. 1, or something close to it, represents their phylogeny accurately. If there were, in fact, a separate lemur stock and a separate loris stock, one should be able to list characters distinguishing the two. It must be assumed that these distinctions are traceable to specific, novel characters developed in one or other stock. Published lists of distinctions are almost exclusively based on living forms^{2,4,10,14}. A summary of the commonest recognized distinguishing characters is given by Weber¹⁵ (see Table 1). Hill¹⁴ gives a far more extensive list, but the same general comments apply.

Such a list should apply to all known lemurs and lorises. In fact, the list is chiefly based on comparison of living Lorisinae (loris or potto) with living Lemurinae (usually *Lemur*). The Galaginae (bushbabies) and Cheirogaleinae (mouse and dwarf lemurs) have rarely been compared with one another, and subfossil lemurs have generally been ignored. When-

ever the distinction between lemurs and lorises is examined with respect to the Galaginae and Cheirogaleinae, the initial clear separation evaporates.

Table 1. LIST OF CHARACTERS DISTINGUISHING THE LEMUR GROUP AND THE LORIS GROUP (from Weber, ref. 15, vol. 2)

Lemuridae	Lorisidae
1. Ring-shaped ectotympanic bone within auditory bulla	1. Ectotympanic bone involved in formation of bulla wall
2. Jugal in contact with the lachrymal bone	2. Jugal separated from the lachrymal bone to varying degrees by the maxilla
3. Os planum of ethmoid bone lacking in the orbit wall	3. Os planum of ethmoid bone incorporated in the orbit wall
4. Ethmoturbinal I small; does not cover the maxilloturbinal	4. Ethmoturbinal I large; covers the maxilloturbinal
5. Internal carotid artery passes through the posterior carotid foramen into the bulla, continues through bulla and enters brain cavity through the basiphosphoid bone	5. Internal carotid artery passes external to the bulla; enters the brain cavity anterior to the bulla, through the anterior lacerate foramen

To take characters from Weber's list (Table 1): in living Galaginae, a ring-like ectotympanic within the auditory bulla has been reported⁵, while our examination of skulls of the common mouse lemur (*Microcebus murinus*) shows that the ectotympanic is sometimes fused to the bulla wall. Van Kampen, usually quoted as the authority on the structure of the auditory bulla in mammals, gives no description of Cheirogaleinae or Galaginae¹⁶. It has also long been known that the Cheirogaleinae show the typical "lorisid" condition in the arrangement of the internal carotid artery and its foramina^{2,14}. Our examination of material in the British Museum (Natural History) and in our own collections shows that in Demidoff's bushbaby (*Galago demidovii*), at least, the relationship between the jugal and the lachrymal is indistinguishable from that in *Microcebus murinus*; in both, the jugal generally contacts the lachrymal. *M. murinus* and *Cheirogaleus major* and *C. medius* have an os planum in the orbit, as Le Gros Clark pointed out in a footnote². Finally, there is no significant difference between *M. murinus* and *G. demidovii* in the arrangement of the ethmoturbinals and the maxilloturbinal in the nose. In both, ethmoturbinal I is large and more or less covers

the maxilloturbinal. Weber's list, and conclusions based on such lists, must therefore be revised.

Similarities between Cheirogaleinae and Galaginae

The vital fact is that the Galaginae were not effectively compared with the Cheirogaleinae in establishing such lists. For example, Pocock¹⁰ did not study *Microcebus* in preparing his monograph, and his conclusions regarding the Cheirogaleinae were apparently based on a small number of male *Cheirogaleus*. Our field studies of *Microcebus murinus* (R. D. M.; Madagascar, 1968) and *Galago demidovii* (P. C.-D.; Gaboon, 1965-69) have shown, however, that there are many ecological and behavioural similarities between these two species:

(1) Both are nocturnal forms, sharing extensive adaptations.

(2) Preferred habitat: fine branch and creeper niche. Locomotion: mixture of horizontal running and vertical clinging and leaping (trees) or hopping (ground).

(3) Diet omnivorous: chiefly fruit and insects. Insects are typically trapped with one or both hands. There is a conspicuous predatory pattern of propulsion by the back legs, which retain their grasp, followed by rapid retraction.

(4) Both species exhibit "urine-washing"—impregnation of both hands and feet with urine, involving a complex behaviour pattern.

(5) Females associate in groups; males are usually solitary. Both sexes usually disperse for feeding. Nests used for sleeping are typically globular arrangements of leaves (interwoven), with a lateral entrance; hollow trees may be used by the mouse lemur. Home ranges around the nests are very stable.

(6) Social and parental interactions include social grooming using the tooth-comb (identical structure in both species) and grasping fur in the hands. Grooming is most common in the head and neck area.

(7) The basic categories of adult vocalizations are identical.

(8) Male home ranges apparently overlap with those of females, thus facilitating reproduction. (When the female is in oestrus, the vulva passes through a cycle of expansion, reddening and opening; during periods of sexual inactivity it is closed.)

(9) The offspring—almost identical in appearance and state of development at birth in the two species—grow slowly and are weaned fairly late. The mother carries the babies in her mouth, not on the fur. Vocalizations of the offspring are generally comparable.

Two major factual conclusions emerge from these comparisons; first, the classical morphological distinctions between lemurs and lorises do not apply to *Microcebus murinus* and *Galago demidovii*, and perhaps do not apply to the Cheirogaleinae and the Galaginae in general; and, second, the two species are extensively similar in ecology, behaviour and general morphology. There are at least four possible interpretations open to discussion: (i) the two species have retained a great number of ancestral features present in all primates before the separation of Madagascar from the African mainland; (ii) convergent evolution for adaptation to a particular ecological niche has produced an enormous number of similarities in the two species; (iii) the Cheirogaleinae and Galaginae are far more closely related than is indicated by Fig. 1 (that is to say, invasion took place across

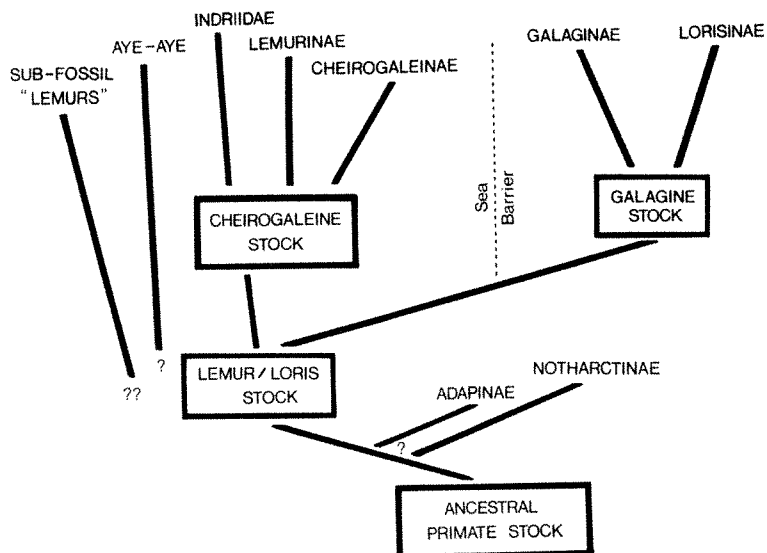


Fig. 2. A provisional outline of possible evolutionary relationships between lemurs and lorises. Note the exclusion of the tree shrews and the uncertain derivation of the subfossil lemurs (*Megaladapis*, *Archaeolemur*, and the like). The post-Eocene "sea-barrier" (= Mozambique Canal) may not have been impermeable.

the Mozambique Canal after the separation of Madagascar); (iv) some combination of (i), (ii) and/or (iii).

Cheirogaleinae and Galaginae may show Ancestral Primate Features

Because many of the similarities between the Cheirogaleinae and the Galaginae seem likely to be ancestral Primate characteristics (or at least ancestral lemur/loris characteristics), it would seem that these two groups have remained very close to the original prosimian condition (though both would, of course, have undergone subsequent specialization). Convergent (or parallel) specialization may have emphasized the similarity based on retention of ancestral features, but the chief source of similarity is probably the retention of ancestral Primate characteristics. This being the case, it is difficult to understand how one can trace the ancestry of the lemurs and lorises to anything remotely like *Plesiadapis* or ancestral tree shrews in the Palaeocene. (It should be pointed out that Simpson¹³ did not compare *Plesiadapis* with either the Galaginae or the Cheirogaleinae.)

We can provide a provisional evolutionary outline (Fig. 2), based on two assumptions: first, that no invasion of Madagascar by African prosimians (or vice versa) took place after the Eocene; and, second, that the similarity between Cheirogaleinae and Galaginae is so pronounced that explanations based exclusively on a convergence hypothesis are unsound. These suppositions may be questioned—indeed, the first, the non-invasion hypothesis, should be very closely examined—but we have attempted to give a clear statement involving wider consideration of lorises and lemurs. One possibility which we are not discussing here is that of diversification of extant and subfossil Malagasy lemurs before Madagascar split off from Africa. There could well have been several lemur types present in the African region during the late Palaeocene, and these might have given rise separately to the rather more extreme Malagasy lemurs (for example, *Daubentonius*, *Megaladapis* and *Archaeolemur*). Only new fossil evidence and extremely detailed comparisons can provide the answer.

The Eocene fossil Notharctidae, widely regarded as direct relatives of the Malagasy lemurs, probably came from a separate stock roughly contemporaneous with the hypothetical lemur/loris stock (Fig. 2).

The extremely provisional diagram of the supposed evolution of lemurs and lorises given in Fig. 2 is, in fact

fairly close to the classification given by Van Valen⁹. It is also in accordance with Romer's approach¹⁷, whereby the lemurs and lorises are regarded as forming one "natural group", rather than two intrinsically separate groups. Significantly, both authors also agree in the exclusion of the tree shrews from the order Primates^{8,17}. It must be emphasized, however, that Fig. 2 is provisional, and that post-Eocene migration across the Mozambique Canal cannot be ruled out.

The study of *Microcebus murinus* (R. D. M.) was supported by a NATO fellowship (SRC, London) and by a grant in aid for field study in Madagascar from the Royal Society. Finance and facilities for the study of *Galago demidovii* (P. C.-D.) were provided by the Mission Biologique au Gabon (CNRS; Professor P. P. Grassé).

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Observations of Clear Air Turbulence by High Power Radar

by

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Clear air turbulence is a hazard to aviation and is thought to have important effects on atmospheric dynamics. This article describes the structure and evolution of clear air turbulence at high altitudes as revealed by a high power radar and vertical soundings of wind and temperature.

THEORETICAL considerations and observations in the free atmosphere suggest that clear air turbulence (CAT) is chiefly a manifestation of Kelvin-Helmholtz (K-H) instability^{1,2}. The nature of K-H instability has been clearly revealed by recent laboratory studies³, and by observations of clouds^{4,5} and of dye-tracers in the oceanic thermocline⁶. K-H instability is a form of dynamic instability produced within a hydrostatically stable flow in the presence of sufficiently strong vertical shear. It appears as amplifying waves ("billows") oriented perpendicular to the shear vector, into which the vorticity is concentrated, and which eventually "break" into turbulent flow on a range of smaller scales. The onset of K-H instability over an atmospheric layer of depth Δz is determined by the Richardson number

$$Ri = (g/\theta)(\Delta\theta/\Delta z)/(\Delta u/\Delta z)^2 \quad (1)$$

where Δu is the vector wind change over the depth Δz and θ is the potential temperature. Ri is a ratio of work done against gravity in vertical motions and the kinetic energy available for disturbances from the shear of the mean flow. Theoretical studies⁷ indicate that $Ri \leq \frac{1}{4}$ is a necessary but not sufficient condition for the onset of K-H instability. For a sheared layer of finite thickness, the spacing of the resulting K-H billows corresponds to the maximum unstable wavelength⁸ given by

$$\lambda = (\pi/g)(\Delta u)^2/(\Delta\theta/\theta) \quad (2)$$

As pointed out by Ludlam⁴, the nature of the bumpiness experienced by an aircraft encountering K-H billows depends on the part of the billow pattern traversed by the aircraft, its heading with respect to the wind shear vector, and also the response characteristics of the aircraft. Ludlam suggests that in the early stages of the evolution of billows the changes in vertical air velocity encountered over horizontal distances equal to Δz might have a magnitude similar to Δu . In some cases this can exceed 20 m s⁻¹.

K-H instability and associated turbulence in the clear atmosphere can be observed with high power radars. This article reviews the principles of radar detection of CAT and then presents some combined radar and radio-sonde observations showing the structure and evolution of

a region of CAT associated with a well defined case of K-H instability.

Radar Detection of Clear Air Turbulence

Studies of the optically clear atmosphere using high power radars with wavelengths of 100 mm and longer have revealed echoes due to incoherent backscatter from inhomogeneities of refractive index⁹. Although in the lower troposphere these inhomogeneities are chiefly due to gradients of humidity, at higher levels they are the result of strong gradients of potential temperature. Clear air radar echoes in the upper troposphere are almost invariably associated with layers of strong static stability within which the potential temperature increases rapidly with height. The presence of turbulence within such layers gives rise to irregularly disposed inhomogeneities of refractive index, and this results in incoherent backscatter from a large number of independent scatterers. Although the inhomogeneities occur over a range of scales, constructive interference occurs only from inhomogeneities separated by half the radar wavelength ($\lambda/2$). Thus the radar effectively singles out the particular Fourier component of the refractive index spectrum that is directed along the beam axis and the wavelength of which is half the radar wavelength.

The scattering of electromagnetic waves by refractively turbulent regions has been treated fully by Tatarski¹⁰ on the assumption that the radar-observed scale is within an "inertial subrange" in which the turbulence is locally homogeneous and isotropic, with an energy spectrum that obeys the Kolmogorov $k^{-5/3}$ law, where k is the wave number. Atlas, Hardy and Naito¹¹, and Ottersten¹², in applying Tatarski's results to the atmosphere, have shown that the radar reflectivity (backscattering cross-section per unit volume) of a refractively turbulent layer is given by

$$\eta = 0.38 C_n^2 \lambda^{-1/3} \quad (3)$$

where C_n^2 is a measure of the intensity of the refractivity fluctuations given by

$$C_n^2 = a^2 M^2 L_0^{4/3} \quad (4)$$

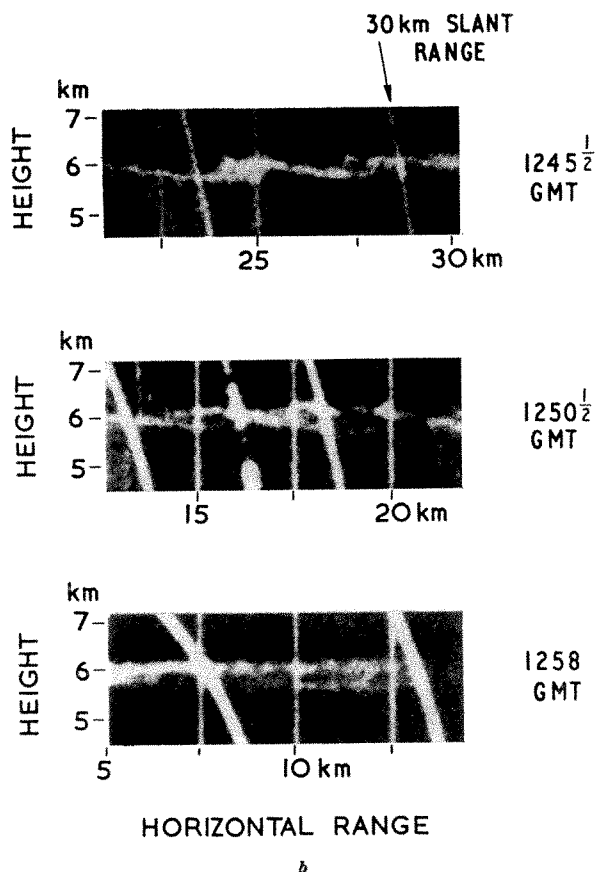
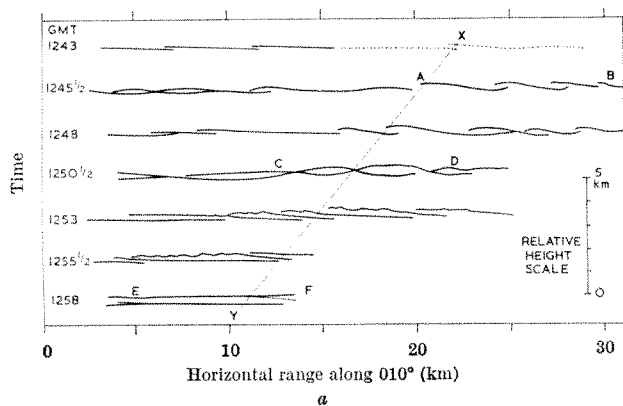


Fig. 1. *a*, Series of tracings of RHI radar photographs at intervals of roughly 2.5 min from 1243 to 1258 GMT showing the development and breakdown of Kelvin-Helmholtz billows on a clear air echo layer. The reflectivity of the clear air layer at 1243 GMT was very weak in the region where it is drawn dotted. Successive tracings are displaced in the vertical by distances proportional to time. Identifiable portions of the train travelled parallel to the dashed line XY. *b*, RHI radar photographs showing the portions of the clear air layer labelled AB, CD, and EF in *a*.

In equation (4), a^2 is a non-dimensional constant, M is the mean vertical gradient of (generalized) potential refractive index, and L_0 is the outer scale of the inertial subrange. For a given vertical wind shear the outer scale is a measure of the intensity of turbulence. Thus C_n^2 , and hence the measured value of radar reflectivity, depends on the intensity of turbulence within the inertial subrange (either at the time of the reflectivity measurement or perhaps a short time earlier).

The outer scale of the turbulence responsible for the radar echo is probably limited by the depth of the echo layer. Recent radar measurements at ultra high resolution of the echo at the top of the planetary boundary-

layer show that this can be very small, typically 10 m (unpublished work of Atlas *et al.*). This order of magnitude is corroborated by refractometer soundings¹², which suggest that a depth of 30 m is typical for the intense refractivity fluctuations at the top of the boundary-layer. Echo layers at high altitudes may be rather deeper; the available measurements, however (including those presented here), are of relatively poor spatial resolution and all that can be said with certainty is that the depth of high altitude layers is often less than 150 m. Thus, although a high reflectivity implies the existence of turbulence on small scales, it does not in itself necessarily imply the simultaneous presence of CAT on the larger scales important in aircraft operations (100–1,000 m). Indeed, on occasions when the clear air echo layers are seen to be level and devoid of structure, they provide evidence of the absence of large scale CAT at least in their immediate vicinity. Frequently, however, clear air echo layers do show irregularities; these are often wavelike or in the form of billows, sometimes with vertical amplitudes of several hundreds of metres. In these circumstances the layer echoes serve as valuable tracers of the larger scale motions which are responsible for the more intense CAT occurrences.

There are two ways of exploiting these tracers to provide insight into the nature of CAT. One is to measure the velocity characteristics of the radar echo by Doppler techniques. The other is to observe the structure and evolution of the perturbed echo layer. The latter approach was first used extensively by Hicks and Angell¹³ and Hicks¹⁴, who obtained photographs showing clear air echo layers perturbed by K-H billows. The billows attained crest-to-trough amplitudes of up to 600 m, across which the total wind change was several metres per second, so that any aircraft encountering them could have expected to encounter significant CAT. Unfortunately no data were obtained showing the life cycle of K-H billows. Atlas *et al.* in their unpublished studies of clear air echo layers have obtained time sequences of data showing successions of K-H billows at different stages of development and breakdown. But their data are Eulerian in the sense that different waves were being observed as they drifted through a fixed radar beam. Thus again it was not possible to interpret the data unambiguously in terms of the life cycle of individual waves. The observations reported in what follows show at least part of the Lagrangian time history (following the motion, that is) of a recognizable patch of CAT associated with K-H billows.

Evolution of a Patch of Clear Air Turbulence

We used the high power S-band radar at the Royal Radar Establishment site at Defford, Worcestershire (2° 09' W, 52° 06' N). The radar consists of a coherent magnetron transmitter and low noise receiver used in conjunction with one of a pair of fully-steerable paraboloid aerials originally designed for radio astronomy studies. Some characteristics are summarized in Table 1.

Assuming the pulse volume to be about half filled, the radar parameters in Table 1 imply a minimum detectable reflectivity η_{\min} of about $5 \times 10^{-15} \text{ m}^{-1}$ at 10 km range (neglecting any advantages gained from photographic integration). One of the chief factors limiting η_{\min} at close ranges is the obscuration caused by backscatter from ground targets detected in the antenna side lobes.

Table 1. CHARACTERISTICS OF THE DEFFORD RADAR

Wavelength	107 mm
Transmitted peak power	~1 MW
Pulse repetition frequency	800 s ⁻¹
Pulse length (in space)	1.3 μ s (200 m)
Total system noise temperature (using a parametric amplifier)	~250 K
Aerial diameter	25 m
Half power beamwidth	0.33°
Maximum azimuth scanning rate	72° per min
Maximum elevation scanning rate	36° per min
Present data recording system	Photography of RHI display

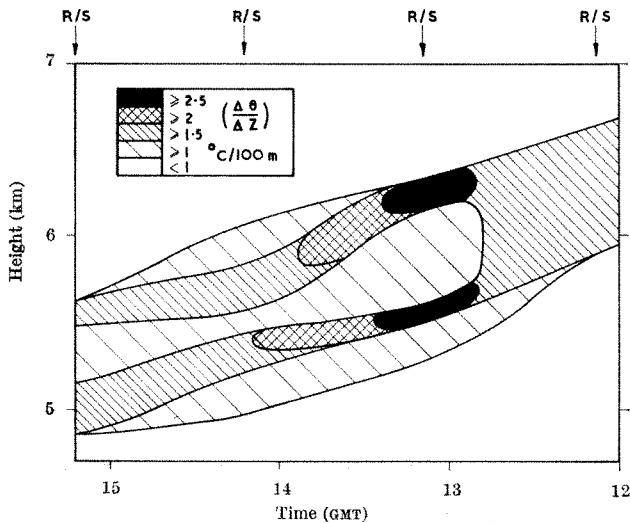


Fig. 2. Time-height pattern of the vertical gradient of potential temperature, based on four radiosonde ascents from Pershore. Note the splitting of the statically stable layer following the occurrence of deep Kelvin-Helmholtz instability.

In the present system this problem is diminished by using (i) a Cassegrain feed to minimize side lobes, and (ii) an MTI system to discriminate against stationary targets.

The normal mode of operation in studies of high altitude CAT is for the radar to be scanned continuously in a vertical section along the direction of the upper tropospheric winds. The received echoes are displayed and photographed on a conventional range-height-indicator (RHI) display. Interpretation of the radar data was aided by special hourly radiosonde ascents from RRE Pershore (8 km north-east of the radar) giving vertical profiles of temperature, humidity and wind speed and direction.

The observations reported here were obtained in cloudless conditions on February 6, 1970, as part of a 10 h sequence of measurements made while a weakening northerly jet was overhead in the upper troposphere. Maximum wind velocities of about 50 m s^{-1} from a direction 010° east of north occurred at a height of about 9 km. Strong vertical wind shears and high static stability occurred both above and below the jet, and clear air layer echoes were detected in these regions throughout much of the observational period. Layer echoes were occasionally perturbed by K-H billows. The full analysis relating the occurrence and structure of the clear air echoes to the temperature and wind shear patterns will be published elsewhere; this article is restricted to a consideration of one of the more clearly documented occurrences of CAT, which took place within a layer of particularly strong shear and stability (that is, a frontal zone) beneath the jet core. At 1243 GMT on February 6 a weak clear air echo was centred within this layer near 6 km; shortly afterwards, as shown by the sequence of radar data in Fig. 1, this echo intensified and was seen to be perturbed by large-amplitude K-H billows.

The radar sections shown in Fig. 1 were orientated along 010° . This direction was within 20° or 30° of both the direction of the wind at the middle of the echo layer (350°) and the direction of the vertical wind shear vector across the echo layer (030° changing to 040° after the occurrence of the billows). Because K-H billows take up an orientation at right angles to the wind shear and travel at the velocity of the winds in the middle of the sheared layer, the orientation of the radar sections was a suitable compromise which revealed practically the true wavelength and which at the same time permitted individual billows to be followed for some time as they approached the radar. Identifiable parts of the train of billows

travelled approximately along the dashed line XY in Fig. 1a. The slope of this line corresponds to a line-of-sight velocity component of 13 m s^{-1} . Pronounced billows were first observed at long ranges (AB in Fig. 1), where their crest-to-trough amplitude was between 300 and 400 m and their wavelength was between 3 and 5 km. Subsequently these same billows were observed to complete their life cycle as they approached the radar, finally giving rise to a double echo layer at 1258 GMT. The detailed evolution of the echo pattern is discussed later.

The radiosonde ascents reveal some of the effects of the intense K-H instability depicted in Fig. 1. The most striking effect (shown in Fig. 2) was the splitting of the layer of strong static stability (that is, large $\Delta\theta/\Delta z$) between the times of the first two radiosondes. Radiosonde No. 1 was launched at 1207 GMT, some 36 min before the first radar observation of K-H billows; radiosonde No. 2 was launched at 1309 GMT, some 11 min after the clear air echo had split into two distinct layers. Both radiosondes ascended through the 6 km level 15 min after launch in a position 15 km due east of the Defford radar. $\Delta\theta/\Delta z$, $\Delta u/\Delta z$ and Ri have been evaluated for both of these soundings over layers 200 metres deep.

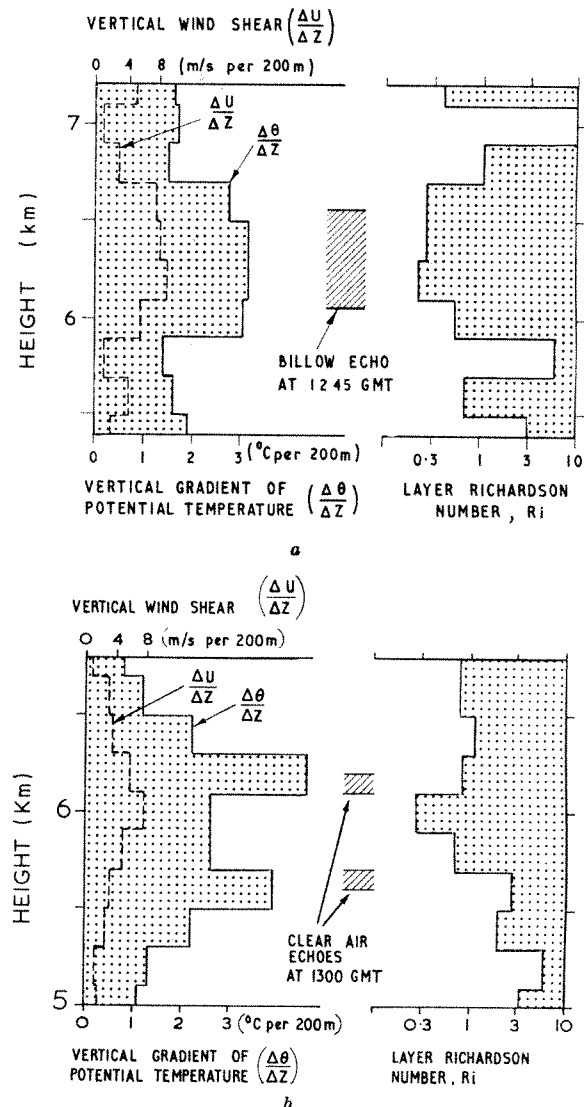


Fig. 3. Profiles of the vertical gradient of potential temperature $\Delta\theta/\Delta z$, the magnitude of the vertical vector wind shear $\Delta u/\Delta z$ and Richardson number, all measured over 200 m height increments. The profiles in *a* and *b* are based on radiosondes No. 1 and 2, launched from Pershore at 1207 and 1309 GMT, respectively. The clear air radar echoes indicated correspond to the vertical extent of the Kelvin-Helmholtz billows at 1245 and to the resulting double layer seen at 1300 GMT.

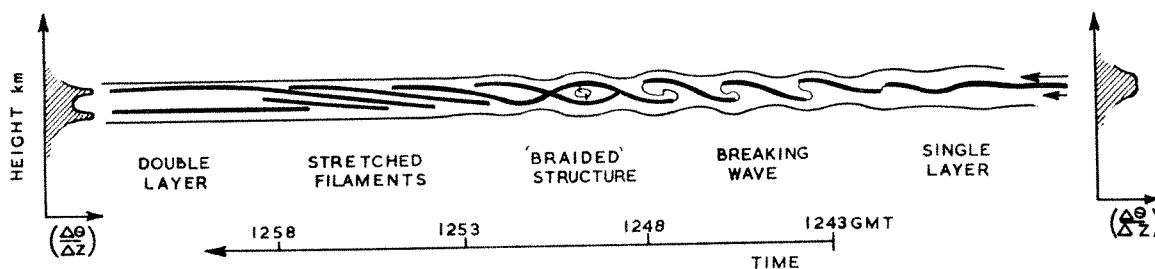


Fig. 4. Schematic representation of the life cycle of an individual Kelvin-Helmholtz billow based on the data in the earlier figures. Time progresses from right to left. Thick lines correspond to the detectable clear air radar echo, which started as a single layer at 1243 and finished as a double layer at 1258 GMT. Schematic vertical profiles of $(\Delta\theta/\Delta z)$ are indicated before and after the occurrence of Kelvin-Helmholtz instability.

The results are plotted in Figs. 3a and b, which show that there was a close correspondence (1) between the initial deep layer of strong static stability and the height of the K-H billows and (2) between the resulting two layers of strong static stability and the height of the double echo layer. (Because there was a small time difference between the radiosonde and radar observations, the indicated heights of the radar echoes in Fig. 3 have been adjusted slightly to take into account the lowering of the overall stable layer). The profiles of $\Delta u/\Delta z$ in Fig. 3 indicate strong shear near the stable layer in both soundings. Although there is no evidence in the second sounding of a split in the shear profile similar to that observed in the stability profile, there was a slight overall decrease (and veer) of the wind shear. As a result the Richardson number, which was between 0.24 and 0.29 over a depth of 600 m before the onset of K-H instability, generally increased (except within a shallow 200 m height interval between the two stable layers).

Interpretation of the Observations

The observations depict several aspects of the evolution and effects of intense Kelvin-Helmholtz instability in the atmosphere. The life cycle of the K-H billows is summarized schematically in Fig. 4, which has been adapted from a diagram by Scorer¹⁵. The starting point, at the right of the diagram, shows a single unperturbed clear air echo layer of limited vertical extent (< 150 m). This was embedded within a deeper (800 m) stable layer across which there was a strong vector wind change (30 m s^{-1} over 800 m, with a 16 m s^{-1} change between 6.1 and 6.5 km). The Richardson number measured over 200 m layers was close to the critical value of $\frac{1}{4}$ over a depth of 600 m for some time before the development of the deep K-H instability. The shallowness and the unperturbed appearance of the echo layer at this time suggest, however, that the intensity of turbulence was then relatively slight.

The layer echo began to show signs of distortion by K-H billows at 1243 GMT. The subsequent development of the pattern of radar echoes associated with the billows can be accounted for in terms of the laboratory observations of Thorpe³. According to Thorpe there is a rapid redistribution of fluid within the dynamically unstable layer as the disturbance grows. At the centre of the rolls there is an accumulation of fluid and a decrease in the gradient of potential temperature; between the rolls there is a sharpening of the gradient of potential temperature. The increased gradient of potential temperature and hence of refractivity in these parts is partly responsible for the more than four-fold increase in reflectivity of portions of the radar echo observed between 1243 and 1248 GMT. Likewise the decreased gradients within the rolls also account for the reflectivity in these parts dropping below the threshold of detectability. By 1250 GMT turbulent mixing initiated within each roll had spread to form cellular (cat's eye) patterns in which the static stability was more nearly neutral. The resulting redistribution of stability was such as to concentrate the stability into two layers, at the top and bottom of the mixed regions¹⁶. These stable boundaries were detected by the

radar, and they produced the so-called braided echo pattern first reported by Hicks and Angell¹³. The final stage in the evolution was for the cells of relatively well mixed air resulting from the breaking of each billow to be stretched out by the wind shear. For a while the radar continued to detect the edges of these regions as sloping filaments of echo, but by 1258 GMT these regions had merged to give a practically homogeneous and horizontal layer 400 to 500 m deep, bounded by two very stable layers detected by the radar as a double layer. (An associated splitting of the wind shear profile was not observed.) Thereafter the double layer of both stability and radar echo persisted for more than 2 h (Fig. 2). Further major splitting did not occur during this time. The distance separating two split layers depends on the amplitude of the billows and on the degree of entrainment across the stable boundaries during the decay of the turbulence⁶. In the present case the final separation was not much greater than the crest-to-trough amplitude of the initial billows. The implied small amount of entrainment is probably a consequence of the strong overall static stability causing the turbulence to decay rapidly.

The vector wind change across the 400 m depth of the billows was shown by the radiosonde data to be as great as 16 m s^{-1} before the onset of the deep K-H instability, and so, according to the criterion of Ludlam⁴, the billow phenomenon almost certainly corresponded to a situation of intense CAT. The present radar equipment is being modified to give Doppler information and when this is achieved it will be possible to obtain a quantitative assessment of turbulence intensity from the radar alone. Thus it is evident that high power radar has considerable potential as a research tool for studying the physical mechanisms associated with atmospheric turbulence and its relationship with larger scale meteorological processes¹⁷. After a long period in which it has been necessary to treat all turbulence statistically (as locally homogeneous, isotropic and stationary), observations by high power radars and by other means are helping to focus attention on ordered motions as the source of intense clear air turbulence.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Polarization-wavelength Profile of the Interstellar 4430 Å Absorption Band

KNOWLEDGE of the scattering and polarizing properties of interstellar grains has now reached the stage where very specific models are being proposed for the composition and size distribution of these grains. It is evident that high resolution studies of the wavelength dependence of interstellar extinction and polarization will provide important tests of the proposed grain models. Although much work has been done on detailed studies of interstellar extinction, the detailed wavelength dependence of interstellar polarization at a higher resolution needs more study. Another related problem concerns the possibility that the diffuse interstellar bands, the most

The Wollaston prism which splits the incident starlight into two beams, polarized in mutually perpendicular planes, was placed in an adjustable mounting above the spectrograph slit. The angle between the emergent rays is 5.8 minutes of arc, corresponding to a separation of 0.3 mm on the plates (Fig. 1). The observations were made on the star 55 Cyg (HD 198478, B3_{1a}, $E_{B-V}=0.57$) at a dispersion of 40 Å mm⁻¹, this star having been studied by one of us¹ for the absorption profile of the 4430 band. The emulsion used was Kodak IIa0. Pairs of orthogonally polarized spectra were recorded for three different positions of the Wollaston prism. In order to obtain corrections for instrumental polarization, the unpolarized star Vega (α Lyr, HD 172167, A0V, $E_{B-V}=0.00$) was observed in the same way. Five pairs of spectra for each star and for each position of the Wollaston prism were obtained.

Calibrations were imposed by the calibration spectrograph associated with the 36-inch telescope. The spectra were measured over the wavelength region 4620 Å to 4380 Å in terms of Baker density, defined as

$$\Delta = \log_{10} \left(\frac{1}{T} - 1 \right)$$

T being the transparency. This function of T is known to be closely linear with magnitude². The spectral resolution is 4 Å, and the slope of the calibration curve was found to be constant over the spectral range measured.

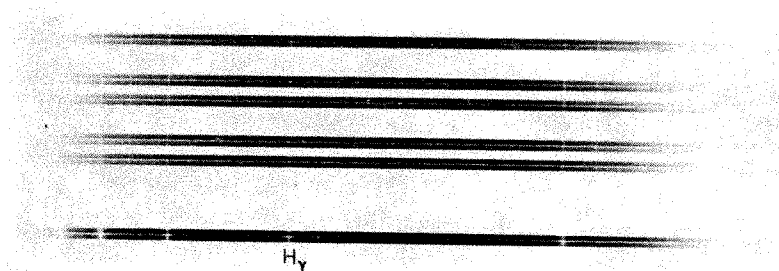


Fig. 1. Pairs of spectra, plane polarized by Wollaston prism.

intense of which occurs at 4430 Å, may be polarized differently from the neighbouring continuum: the amount and nature of this difference, if any, may give a clue to their origin. Spectrophotometry of these bands is always difficult because of the width and shallowness of the features, and polarization studies are correspondingly more difficult. By the use of a Wollaston prism at the Cassegrain focus of the 36-inch telescope of the Royal Observatory, Edinburgh, we have been able to derive a polarization profile across the 4430 band. This report describes the method and presents the preliminary results.

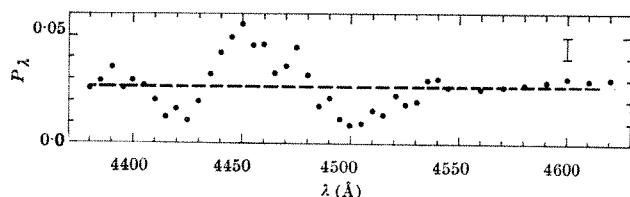


Fig. 2. Wavelength dependence of the degree of interstellar polarization across the 4430 Å absorption band. The broken line indicates the mean value for the spectral region away from the band.

Because Vega is unpolarized, the pairs of spectra should be identical. Any difference would indicate the presence of instrumental polarization which, of course, must be taken into account to interpret the results of 55 Cyg. It is found that the differences $\delta\Delta$ between the pairs of spectra for Vega are linear with $1/\lambda$ and have a small inclination with the abscissa, indicating that instrumental polarization is present, although small, and those for 55 Cyg show signs of an underlying profile. To evaluate the degree of polarization we found the difference of the values of $\delta\Delta$ for 55 Cyg from the corresponding values for Vega. These differences were multiplied by the slope of the calibration curve to derive the monochromatic magnitude differences.

For partially plane polarized starlight, the magnitude difference Δm_γ between the two orthogonally polarized spectra in each pair is given by

$$0.46 \cdot \Delta m_\lambda = P_\lambda \cos 2\varphi \quad (1)$$

where P_λ is the degree of polarization, and

$$\varphi_\lambda = \alpha - \theta_\lambda \quad (2)$$

where α and θ_λ are the direction of polarization of the Wollaston prism and the position angle of interstellar polarization, each measured with reference to a sky oriented axis.

Let $\Delta m'_\lambda$ be the corresponding magnitude difference when the Wollaston prism is rotated by an angle x . P_λ and φ_λ are then given by

$$\varphi_\lambda = \frac{1}{2} \tan^{-1} \left(\cos 2x - \frac{\Delta m'_\lambda}{\Delta m_\lambda} \right) / \sin 2x \quad (3)$$

$$\text{and} \quad P_\lambda = 0.46 \cdot \frac{\Delta m_\lambda}{\cos 2\varphi} \quad (4)$$

The results are presented in Fig. 2, which shows the detailed wavelength dependence of the degree of polarization in the spectral region from 4620 Å to 4380 Å. In the continuum away from the 4430 feature, the degree of polarization is 2.7 per cent; we see, however, that this changes across the spectral region affected by this feature, the maximum polarization being observed at $\lambda \sim 4450$ Å. There is no significant variation of the position angle θ within the measured wavelength range, its numerical value in equatorial coordinates being $3^\circ \pm 10^\circ$.

Coyne and Gehrels³ have measured the polarization of 55 Cyg at several wavelengths in the range 0.3–1.0 μ m. The percentage polarization that they derived is $P_v = 2.68$ at $v = 4292$ Å and $P_r = 2.89$ at $\lambda = 5128$ Å, and the position angles in equatorial coordinates are $1^\circ.4$ and $2^\circ.0$ respectively. The present results are in adequate agreement (see Fig. 2). The fact that the polarization changes over the spectral region of the 4430 interstellar band implies that the band is polarized differently from the continuum, and the 4430 process involves polarization as well as extinction. This conclusion then indicates that the explanation of the 4430 process in terms of gaseous absorption may not be tenable.

We would like to emphasize, however, that these results are preliminary and have to be confirmed by further observation. An extensive programme of measuring the detailed wavelength dependence of interstellar polarization is under way.

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Galactic Component of the Diffuse X-ray Background

Cooke, Griffiths and Pounds¹ have recently detected an increase of intensity of the cosmic X-ray background in directions close to the galactic plane, indicating the possible existence of a diffuse galactic X-ray component. The similarity of this galactic component, in both intensity and spectrum, to the general isotropic background would tend to suggest a possible causal connexion. This report argues that interstellar dust occurring near the galactic plane would scatter the isotropic background so as to produce the observed phenomenon.

The existence of a diffuse background of optical radiation close to the galactic plane has been known for many years and is usually attributed to the scattering of starlight by interstellar grains²⁻⁴. The same grains would also scatter X-rays from a cosmic background as well as from

discrete sources. For grains of radius a the scattering cross-section σ for X-rays of wavelength λ is defined by the equations^{5,6}

$$\left. \begin{aligned} \sigma &= Q(\rho) \pi a^2 \\ \rho &= 2N \left(\frac{e^2}{mc^2} \right) a \lambda \\ Q(\rho) &\cong 2 \text{ if } \rho \gg 1 \\ Q(\rho) &\cong 2\rho^2 \text{ if } \rho \ll 1 \end{aligned} \right\} \quad (1)$$

Here e, m are the electron charge and mass, and N is the total electron density in the material, $\sim 10^{24} \text{ cm}^{-3}$ for most solids. For grains of radii $\sim 3 \times 10^{-5} \text{ cm}$ the cross-section σ given by the formulae varies from $\sim \pi a^2$ to $\sim 0.01 \pi a^2$ over the waveband observed by Cooke *et al.*¹ ($\sim 6 \text{ Å} - 0.6 \text{ Å}$); and for grains of radii $\sim 3 \mu\text{m}$ the cross-section remains close to $\sim \pi a^2$ over the same waveband. The phase function for the scattering of X-rays is very strongly forward throwing, the scattering being almost entirely directed into a cone of semi-angle $\sim \lambda/2a$ (ref. 6).

At optical wavelengths the extinction coefficient of the interstellar medium is ~ 2 magnitudes per kpc in directions close to the galactic plane. Because the optical extinction efficiency of grains could be high ($Q \approx 4$ in some cases⁷) it follows from our earlier remarks that the X-ray scattering coefficient of the interstellar medium could be as high as

$$\kappa_{\text{scat}}(6 \text{ Å}) \sim 1 \text{ magnitude kpc}^{-1} \quad (2)$$

at $\lambda \sim 6 \text{ Å}$. At the shorter wavelengths, $\lambda \sim 0.6 \text{ Å}$, the scattering coefficient is likely to be dominated by the relatively small fraction of 1–3 μm grains that may be present. If such grains contribute ~ 10 per cent to the optical extinction it is likely that

$$\kappa_{\text{scat}}(0.6 \text{ Å}) \sim 0.1 \text{ magnitude kpc}^{-1} \quad (3)$$

The mean X-ray absorption cross-section of the interstellar gas per H atom is (ref. 8)

$$\sigma_E \approx 4 \times 10^{-22} (\lambda/12.4 \text{ Å})^{8/3} \text{ cm}^2 \quad (4)$$

With a mean atomic hydrogen density $\sim 1 \text{ cm}^{-3}$ the X-ray absorption coefficient is thus

$$\kappa_{\text{abs}} \sim (\lambda/12.4 \text{ Å})^{8/3} \text{ magnitude kpc}^{-1} \quad (5)$$

From relations (2), (3) and (5) it is clear that the albedo of interstellar matter is close to unity throughout the X-ray waveband observed by Cooke *et al.*¹

Following Henyey and Greenstein² I consider an infinite plane parallel slab model of the Galaxy of total X-ray optical depth $2\tau_1$ with the Sun situated in the mid-plane. Let I be the intensity of unscattered diffuse cosmic X-rays, assumed constant at every point in space. The intensity of once-scattered X-rays arriving at the location of the Sun from a direction making angle θ with the galactic plane is

$$I_1(\theta) \cong \gamma(1 - e^{-\tau_1 \cos \theta}) \int \Phi d\omega \quad (6)$$

where γ is the albedo and Φ is the phase function for scattering normalized so that

$$\int \Phi d\omega = 1$$

Assuming I to be approximately isotropic, equation (6) gives

$$I_1(\theta) \approx \gamma(1 - e^{-\tau_1 \cos \theta}) I \quad (7)$$

as a first order solution to the scattered component of the diffuse X-ray background. (The contribution to I from discrete sources has been neglected here, but the data currently available on sources suggest that its inclusion will not alter our general conclusions.)

From relation (7) with $\gamma \approx 1$ we obtain $I_1(\theta) \approx I$ in directions close to the galactic plane, as observed by Cooke *et al.*¹. The direction for which $I_1(\theta)$ falls to $\sim I(0)/2$ is defined by $\theta \approx 2\tau_1$, so that the observed angular extent of the galactic background $20 \lesssim 6^\circ$ is obtained if $\tau_1 \lesssim 0.03$ at X-ray wavelengths. Because we have $\tau_1 \approx 0.1$ at optical wavelengths^{2,3} it is likely that a value $\lesssim 0.03$ may be

appropriate for the X-ray region relevant to the present discussion. From relation (7) it is also clear that the wavelength dependence of I_1 must be closely similar to that of I for directions very close to $\theta \sim 0$. Because τ_1 is likely to decrease with increasing energy, the present model predicts that the angular spread of the diffuse galactic X-ray background decreases with increasing photon energy.

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Electron Correlations and Solar Neutrino Counts

EXPERIMENTS to detect the neutrino flux from the Sun could be used to test the basic hypotheses concerning the internal constitution of the Sun and the nuclear fusion reactions believed to be occurring in its interior. The measurements of Davis, Harmer and Hoffmann¹ give an upper bound on the neutrino counting rate which is approximately half of the theoretical rate calculated by Bahcall, Bahcall and Shaviv², and an order of magnitude smaller than theoretical rates obtained by other authors. Before the discrepancy between theory and experiment can be accepted as requiring a radical change in the theory of stellar structure, it is first necessary to reduce the uncertainties and poor approximations contained in the "constitutive relations" of the theory; that is, in the equations for the nuclear reaction rates, the pressure and the opacity³, each expressed as a function of density, temperature and chemical composition of the medium. In current tables⁴ of solar opacity, the contribution from the scattering of radiation by free electrons is calculated under the unjustified assumption that the plasma of the solar interior can be treated as a perfect classical gas, and hence that the scattering cross-section is given simply by the classical frequency-independent Thomson cross-section $N_e\sigma_t$, for N_e independent electrons in the plasma. This report shows that corrections to σ_t produced by classical⁵⁻⁷ and quantum⁸ electron correlations reduce the electron-scattering opacity in the core of the Sun by approximately one-third, and that this leads to a significant reduction in the theoretical neutrino flux.

The percentage deviations from σ_t resulting from electron correlations (classical plus quantum corrections) in the variable-composition model of Bahcall *et al.*² are given in Table 1. The domain considered extends from the centre out to 0.33 of the solar radius, and contains 0.69

of the solar mass and the energy generation region. The quantity b is $\lambda_i/(4\pi\lambda_{De})$ where λ_i is the wavelength of radiation at the black-body maximum; the classical plasma parameter is

$$\Lambda = \beta e^2 / \lambda_D \simeq 2\pi^{1/2} e^3 \beta^{3/2} n_j^{1/2} (3+X)^{1/2} (1+X)^{-1/2}$$

where $\lambda_D^2 = 4\pi\beta e^2 \sum n_j Q_j^2$, and Q_j is the charge on species j (the term with $Q_j = -1$ defines λ_D^2); and the cross-sections $\delta\sigma$ are straightforward generalizations to a multi-component system of those given previously⁶ for pure hydrogen models. In performing the integration over solid angle to obtain total cross-sections I have not inserted the weight factor $(1 - \cos \theta)$, where θ = scattering angle, suggested by Watson⁷, because no proof has been given that the factor is necessary for the case of a non-relativistic system of scatterers. The effect of correlations has been taken into account to order (e^2, z^2) in perturbation theory, where $z = \exp(\beta\mu)$ is the fugacity and μ the chemical potential of the electrons. It is seen that the system falls neatly into the near-classical domain^{8-10,21} of the statistical mechanics of interacting particles. At the centre the quantum parameter $\zeta = 4\pi^{3/2} n_e \lambda^3$ reaches its maximum value permitted by the small z approximation. Because this value is somewhat larger than that considered previously⁶, however, the shift in fugacity resulting from quantum statistics and interactions should be included. For simplicity of computation I have taken $z \simeq z_0(1 - \Lambda/2 - \zeta(\beta e^2/\lambda)/(2\pi)^{1/2})$, where the second and third terms on the right-hand side are, respectively, approximations to the first-order direct and exchange corrections to the quantum ideal gas fugacity $z_0 = e^{\beta\mu_0} \simeq \zeta(1 + \zeta(2(\sqrt{2} - \zeta))^{-1})$. At the outer limit of the solar region considered the quantity $\beta e^2/\lambda$ is still within the approximate upper bound of 0.35 suggested by the calculation¹¹ of the zero-range pair correlation function for arbitrary values of $\beta e^2/\lambda$ in an ionized system. Because $\Lambda \ll 1$, the Debye term $\delta\sigma_D$ gives a good approximation for the classical correlations. The zero-order exchange term $\delta\sigma_0^x$ has been calculated by expanding the London-Placzek¹² pair correlation function, with the appropriate spin factor, to order z^4 .

The total result is to reduce the effective cross-section by an amount which is almost constant over the whole solar domain considered, and which is equal to 34.5 per cent at the centre. Consequently, it can be seen from the energy transport equation¹³ that the temperature gradient in the core is reduced, and because the rates of nuclear reactions are highly sensitive to the value of the ambient temperature, a non-negligible modification to the neutrino flux is expected. A bonus side-effect of the reduction in temperature gradient is that the assumption that energy transfer in the core occurs by radiation rather than convection becomes more plausible. An estimate of the modification to the neutrino flux follows immediately from the work of Bahcall, Bahcall and Ulrich¹⁴. These authors computed the effect on the solar model of Bahcall *et al.*² of hypothetical localized deviations in the opacity κ , given by

$$\kappa(\text{perturbed}) = f(T)\kappa(\text{standard})$$

where $f(T) - 1 = \alpha\{1 + 100((T - T_0)/(T + T_0))^{2.1} - 1\}$ is an arbitrary smooth function of T which reaches a peak value of

Table 1. PERCENTAGE DEVIATIONS FROM THE THOMSON CROSS-SECTION

T ($\times 10^6$)	ρ (g cm^{-3})	X	Λ ($\times 10^{-2}$)	ζ ($\times 10^{-1}$)	$\beta e^2/\lambda$	b	$\delta\sigma_D$	$\delta\sigma_0^x$	$\delta\sigma_0^{ex}$ (Percentages) ¹	$\delta\sigma_{cl}$	Total
14.9	150	0.415	5.2	2.28	0.21	0.58	-30.1	-6.97	1.15	2.05	-34.5
14.5	136	0.454	5.2	2.21	0.21	0.58	-30.4	-6.77	1.13	1.93	-34.7
13.1	99.0	0.575	5.3	2.03	0.22	0.60	-31.5	-6.23	1.09	1.60	-35.7
11.9	76.7	0.649	5.4	1.90	0.23	0.62	-32.4	-5.85	1.08	1.42	-36.4
10.0	48.2	0.725	5.6	1.62	0.25	0.66	-33.1	-5.02	1.01	1.21	-36.6
8.56	30.8	0.753	5.7	1.33	0.27	0.67	-32.9	-4.15	0.90	1.07	-35.8
6.91	15.9	0.764	5.6	0.95	0.30	0.66	-32.1	-3.01	0.73	0.89	-34.1
5.84	8.95	0.766	5.5	0.69	0.33	0.64	-31.0	-2.22	0.58	0.74	-32.5

ρ = Mass density, X = mass fraction of hydrogen, $\lambda = h/(\beta/2m)^{1/2}$, $\beta = (k_B T)^{-1}$.

α at $T=T_0$ (variable). We compare the hypothetical deviation $f(T)-1$ for the case $T_0=14\times 10^6$ K and $\alpha=-0.2$ with the real deviation obtained from Table 1 by assuming that the electron-scattering process contributes one-third of the total opacity after correlations have been taken into account. Then, if electron correlations are the only source of deviation from the standard values of core opacity, one can immediately estimate a reduction in the value of the theoretical neutrino counting rate of 30 to 40 per cent, and a fair match between theory and experiment would be obtained. Recently, however, increases have been found for the opacity contributions from autoionization lines¹⁵, ordinary lines¹⁶ and bound-free transitions¹⁶. The first two increases need not disturb us overmuch because they are only appreciable at relatively low temperatures. In the latter case, however, uncertainties^{17,18} in the accepted photospheric abundance of iron leave open the possibility that a large increase in opacity in the core might be obtained from the bound-free absorption of radiation by highly ionized iron¹⁹, assuming that photospheric and core abundances of iron are related. Such a result could more than offset the decrease in neutrino rate resulting from electron correlations. It is clear now that before one can talk seriously of "predicting" the solar neutrino flux, the details of the elementary physical processes contributing to the opacity, as they occur in a real plasma at the appropriate density and temperature, have to be understood. Together with some uncertainties in neutrino production rates and absorption cross-sections¹⁴, the major unknown would then be the chemical composition of the core, and a more realistic estimate of the latter might be obtained by considering gravitational separation of the elements of high atomic number²⁰. Although the neutrino rate seems to be two or three times more sensitive to hypothetical deviations in the equation of state¹⁴ of the solar core than in the opacity, the actual deviations in the equation of state are far smaller than the hypothetical ones ($\alpha=\pm 0.1$), provided (and this may not be true²⁰) electron correlation effects are the only omissions. Because Bahcall *et al.*¹⁴ have already taken into account the zero-order exchange and classical Debye term in their "standard" equation of state, the principal term remaining is the first order exchange¹⁰ term $\simeq -\pi n_e \lambda^3 (\beta e^2/\lambda) \simeq -0.005$ (the classical ideal gas contribution is unity), at the "sensitive" temperature¹⁴ of 8×10^6 K. The uncertainty-principle term²¹ is of order e^4 , and can be neglected.

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Lateral Extension in the East African Rift Valleys

MCKENZIE *et al.*¹ have proposed a pole position and rotation angle to describe the opening of the East African Rift Valleys. On the basis of this rotation, they expect extensions of about 30 km in Kenya and 65 km in northern Ethiopia to have taken place. It follows that the mean relative motion of the two plates in Kenya should have been in a direction of 124° east of north. Interpretation of the gravity anomalies from the Kenya Rift provides some confirmation of the hypothesis.

Over the floor of much of the rift valley in Kenya there is an axial, positive Bouguer anomaly²⁻⁵. I have made a detailed interpretation (ref. 5 and unpublished) of this positive anomaly between 0.5° N and 1.5° S. Calculation suggests that the anomaly is caused by dense intrusions beneath the rift floor, presumably of material derived from the upper mantle, which have filled the gap between the separating continental blocks. The total calculated width of these intrusions is between 16 km and 28 km, depending on the assumed density contrast and its variation with depth. If the intrusions represented the total extension, between 2 km and 14 km of the expected extension would be unaccounted for. An additional extension of about 5 km could, however, be taken up by normal faulting⁶. Consequently, the amount of extension inferred from the gravity anomalies is in agreement with that predicted from plate tectonics.

It is also possible to deduce from the gravity anomalies the approximate direction of the relative motion vector. In southern Kenya, the rift follows an S-shaped course: from 2° N to 0.5° N, and south of 1.0° S, its trend is approximately 20° east of north; between 0.5° N and 1.0° S, the trend is about 20° west of north. It is assumed that over this small area the Earth's surface can be considered flat, and that the direction of relative motion is constant (the rotation angle is only 1.9° , so this is approximately true). When a flat plate is broken along an irregular line (the rift) and the two parts are separated without rotation, the amount of separation measured at any point perpendicular to the rift direction varies as the sine of the angle between the local rift direction and the relative motion vector. Because the relative motion across the Kenya Rift near the equator is expected to be approximately 124° east of north, those sections of the rift which trend east of north should show greater extension than those trending west of north. The positive Bouguer anomaly is indeed wider in the NNE-trending sections of the rift, and a quantitative study of the variation of the width of the inferred intrusions with changes of direction of the rift suggests that the direction of relative motion is about 120° east of north. This is in good agreement with the direction of the small circles about the proposed rotation pole; however, considering the inherent ambiguity of any gravity interpretation, and the probability that all of the extension is not taken up by intrusion, this result should be viewed with caution.

Finally, it is known that there are positive Bouguer anomalies over the floor of the main Ethiopian Rift⁷, but at present there are insufficient data to enable the amount of extension to be estimated. A detailed gravity survey of this area is now being made by the Geophysical Observatory in Addis Ababa, and it is hoped that the results

will enable the extension in the Ethiopian Rift to be determined.

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The Great Glen Fault in the Shetland Area

THE Bouger anomaly map of the continental shelf to the west of Orkney and Shetland recently published by Bott and Watts¹ provides gratifying confirmation of the geology of this area which I proposed earlier². My predictions were based on an interpretation of the aeromagnetic map of the area and include not only most of the deep sedimentary basins proposed by Bott and Watts but also the great NW-SE trending strip of Lewisian rocks labelled *A* by Bott and Watts.

The chief difference between the gravity map¹ and my interpretation map based on the aeromagnetic map of the area concerns this anomaly *A*. On the gravity map anomaly *A* continues across the north of Shetland to about 0° 40' W. The equivalent feature on the aeromagnetic map ends to the east of 1° 00' W.

After consideration of both the geological evidence and of the aeromagnetic map I correlated the Great Glen fault with a very powerful fault of transcurrent type exposed in Shetland and called the Walls Boundary fault. In conformity with the pattern of the aeromagnetic map I extended the Walls Boundary fault north of Shetland along a line striking only slightly east of north. This extrapolation takes it through part of Bott and Watt's anomaly *A*. Because their anomaly is not displaced along this line they suggest that the Great Glen fault must pass to the east of Shetland and not through it.

This suggestion completely ignores the known geology of the area; it fails to take the Walls Boundary fault into account. This fault has all the hall-marks of a great transcurrent fault. It must continue to the south and north of Shetland for considerable distances. There is nothing on the aeromagnetic and gravity maps to set a limit to the extension of the Walls Boundary fault to the south. On both maps the line of best fit for it is a smooth curve from Shetland to Inverness passing close to the west of Fair Isle.

The extrapolation of the Walls Boundary fault to the north of Shetland can be made with less certainty. The line which best fits both the gravity and the aeromagnetic maps lies to the east of my original line². It passes through 1° 00' W and 61° 00' N and skirts the eastern end of gravity anomaly *A*.

With this projected line for the fault the nearly N-S strike of the fault in Shetland becomes a local deflexion of strike from the general NNE strike to the north and south of Shetland. The change from concave west to concave east can be seen in Shetland, where the fault is

intermittently exposed over 59 km. For 31 km after it enters Shetland from the south the fault has a strike of 004°. For 28 km before it leaves Shetland to the north the strike is 011°. The maximum strike necessary to carry it past anomaly *A* is 020°.

This configuration for the fault in the Shetland area has the advantage that the Nesting fault (a 16 km displacement transcurrent fault (2)) and other major parallel faults in Shetland become the *en echelon* branches which would be expected if such a deflexion of strike took place.

I maintain that Bott and Watts's gravity map not only confirms the presence of the deep sedimentary basins and crystalline gneiss ridges on the shelf to the west of Shetland which I predicted² but also provides additional evidence of the connexion between the Walls Boundary fault and the Great Glen faults and shows that to the north of Shetland the fault resumes the strike it had to the south of Shetland.

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WE apologize to Dr Flinn for any disquiet we may have caused by omitting to refer to his recent paper¹. We agree with him that the aeromagnetic map² distinguishes regions of shallow and deep magnetic basement by the smoothness of the contours. We disagree, however, with his use of the general anomaly level as an indication of sediment thickness, which is the distinction between his pattern *A* anomalies which "probably overlie deep sedimentary basins" and pattern *B* which "covers sedimentary basins less deep than those of pattern *A*". The usual geophysical approach is to estimate the depth to the basement using well established quantitative techniques³. One of the main points of our report⁴ was to present evidence for three deep Mesozoic basins marked *C*, *D* and *E* on our map. These basins are not delineated in Flinn's interpretation, although they do occur within wider tracts which he interprets as sediment covered.

Flinn has suggested a new line for the Great Glen fault which does not cross gravity "high" *A* north of the Shetlands. This raises a problem which needs to be recognized.

To quote Allen⁵, "probably the most impressive feature of thoroughgoing transcurrent faults is their extreme linearity over literally hundreds of kilometres". Large transcurrent faults should now be interpreted as transform faults which play an essential part in the scheme of global tectonics as boundaries between plates of lithosphere moving laterally relative to each other⁶. If there is no significant internal deformation within the plates, the fault plane must lie on a small circle with reference to the pole of rotation about which the relative motion can be described. The trace of the fault plane can only deviate from a small circle if either one or both of the plates suffer internal deformation during the movement, or if the fault plane has been horizontally displaced at a later stage. Thus Benioff⁷ interpreted the bend in the San Andreas fault in terms of sinistral movement on the Garlock fault which started to develop later than the San Andreas, accompanied by severe distortion where they intersect. The bends of the Alpine fault of New Zealand⁸ can be interpreted in terms of splaying of the fault and deformation currently occurring near the bends.

The new line of Flinn involves changes in direction of the fault south and north of the Shetlands. The bend to the north is particularly sharp. Flinn's line would be acceptable if it could be demonstrated that the required

contemporaneous or later deformation had occurred in the vicinity of the postulated bends. Such deformation might possibly fit into the late Caledonian movements. This problem would be more acute for significant Tertiary (or even post-Devonian) crustal shortening or extension affecting north Britain. The known Tertiary movement is predominantly relatively minor regional extension involved in dyke injection, basin formation and normal faulting.

Collette^{9,10} has presented further evidence to suggest that the line of the Great Glen fault lies east of the Shetland Isles. It is clear that all hypotheses give rise to problems and this should encourage us to look for further evidence particularly in the vicinity of the postulated bends. Perhaps the simplest is the hypothesis advanced by Pitcher¹¹ that the Walls Boundary fault is a splay of the main fault. The main fault would then pass east of the Shetlands.

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Structure Determination by the Combination of Anomalous Scattering and Direct Methods

PHASE determination by direct methods is more difficult for non-centrosymmetric than for centrosymmetric crystals. The phase, $\alpha(h,k,l)$, can take any value for the former crystal between 0 and 2π while the choice for the latter crystal is limited to two values, 0 or π . If the structure is non-centrosymmetric and contains a small number of atoms which scatter anomalously, then $\alpha(h,k,l)$ can be determined from the Bijvoet difference, for example, $\Delta I = |F(h,k,l)|^2 - |F(\bar{h},\bar{k},\bar{l})|^2$, and the known phase α'_p of the anomalous scatterers^{1,2}. From

$$\alpha'_N = \alpha'_p + \pi/2 \pm \theta \quad (1)$$

and

$$\cos \theta = \frac{\Delta I}{4 |F'_N| |F'_P|} \quad (2)$$

where α'_N is $\alpha(h,k,l)$, the phase of the reflexion, F'_N , if there were no anomalous scattering. F'_P is the contribution from the absorption term $\Delta f'_P$. The indeterminacy in equation (1) arises because the cosine is an even function. In practice, the ambiguity has been resolved by choosing α'_N to be the phase closest to α'_p (ref. 3) or by calculating a double phased synthesis². An alternative, indeed the original method, is to calculate the Patterson sine function⁴.

A different approach⁵ is to recognize that the incorporation of anomalous scattering data reduces the problem to the comparative simplicity of the centrosymmetric case, or to a choice between two phases, and then to use direct methods to select the observed phase closest to the

correct value. An important difference is that the two possible phases α_1 and α_2 need no longer be π apart.

When $|\cos \theta|_{\text{obs}} \geq 1$ then $\alpha_1 = \alpha_2$ and the phase is known unambiguously. Such reflexions form a basic set from which the phase determination may proceed. For L(+)-lysine hydrochloride dihydrate, using Raman's³ data, fifty-two out of 128 phases can be determined unambiguously in this way. Similarly, from the neutron diffraction data for cadmium nitrate tetradeuterate⁶, thirty-two out of 161 phases can be determined.

From the basic set, there are two possible ways of proceeding. Phases for the remaining reflexions can be calculated and the observed phase closest to the calculated phase can be chosen or, alternatively, phases can be determined and refined without paying further attention to the observed phases. A reasonable approach would be to carry out sufficient cycles of the first alternative to determine as many phases as required, and then to refine them by the second method, because the observed phases will be liable to error. A preliminary cycle in which the basic set alone is refined should be performed because α_1 for the set may have only a small number of discrete values depending on the space group and the number of anomalous scatterers in the asymmetric unit. For example, for lysine hydrochloride, the phases of the basic set were 90° or 270° . It should be noted that no arbitrary origin-defining phases can be used because the origin is already determined when placing the anomalous scatterers.

The method described here could be useful for determining the structures of large molecules of biological interest which are often solved by combining multiple isomorphous replacement with anomalous scattering data. An obvious advantage would be in the reduction of the number of data to be collected.

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Supralinearity of Thermoluminescent Phosphor Lithium Fluoride

THE non-linear response to dose reported for many thermoluminescent phosphors^{1,2} has caused serious concern both for dosimetry and for dating techniques.

We have investigated square samples of TLD-100 phosphor 3 mm across and 1 mm thick. The thermoluminescence output induced by a ⁹⁰Sr source was measured conventionally with a photomultiplier tube in oxygen-free nitrogen and a heating rate of 20°C/s . A dose of a few kilorads was given to the phosphor and the TL output was measured. In addition to the peaks reported by Zimmerman *et al.*³, a tail was observed at the higher temperature region. A detailed study on this tail showed that there are at least two peaks in its fine structure. These two peaks were found to increase with dose to saturation at near 3 megarads. The peak positions are at 370°C and 430°C respectively. The structure of this tail and its dependence on dose can be seen from Figs. 1 and 2.

A thermal annealing experiment was performed in order to study the characteristics of these peaks. Both peaks were found to tail off exponentially at constant

temperature. The dependence on annealing temperature of the half-lives of both peaks was found to be the same as for the peaks reported by Zimmerman *et al.*³ With the same annealing technique, however, the *s* factors and the energy depths of the two peaks were found not to fit the model of Randall and Wilkins⁴. These facts suggested that the peaks are not related to the electron trapping centres. An optical absorption measurement on an irradiated phosphor showed that the two peaks are related to the absorption V-centre of 250 nm, which is also present in a virgin TLD-100 phosphor but in smaller amounts. Heating the phosphor up to 300° C and then rapidly quenching to room temperature can remove the absorption F-centre of 444 nm completely, but the V-absorption peak is only reduced by a small factor. It was also found that the position of this V-absorption peak shifted to a shorter wavelength region on quenching at a higher temperature. This coincided with the shift of the two peaks to a position equivalent to a higher quenching temperature on annealing at a higher temperature or with longer annealing time at constant annealing temperature. The absorption spectrum of an irradiated phosphor and the change due to different quenching temperatures are shown in Fig. 3.

The height and area of these two peaks were found to increase linearly with dose in the region of a few kilorads. The striking fact is that, by extrapolating to zero glow, there is a threshold dose for producing these

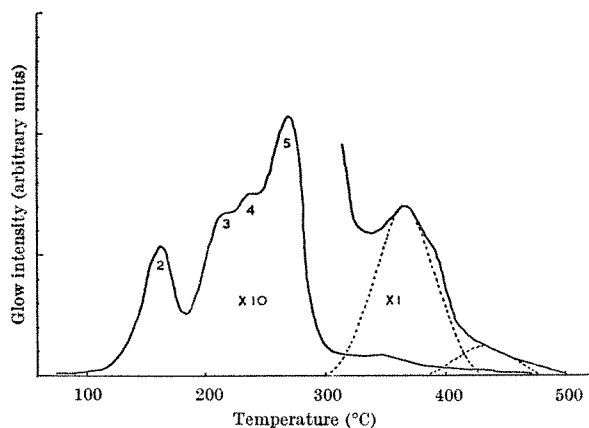


Fig. 1. Thermoluminescent glow of TLD-100 phosphor induced by a dose of 30 krad (⁹⁰Sr source).

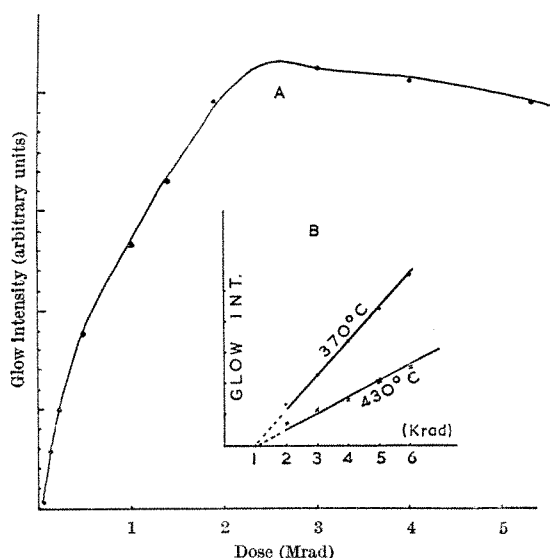


Fig. 2. Thermoluminescent glow against β radiation dose for TLD-100. A, 370° C + 430° C peak area; B, individual peak height.

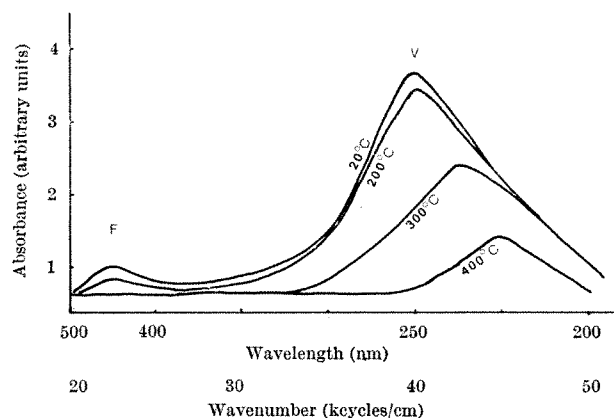


Fig. 3. Optical absorption spectrum of an irradiated TLD-100 phosphor (5.4 Mrad) for different quenching temperatures.

peaks by radiation. The threshold dose was found to be 1 kilorad, which coincides with the dose at which supralinearity is reported to begin in TLD-100 phosphor⁶. When the phosphor was exposed to a high dose of a few hundred kilorads we can see that the 2nd, 4th and 5th peaks reported by Zimmerman are all saturated, but the 3rd peak is still increasing with the two high temperature peaks. This fact suggests that extra light should be expected as more recombination centres are available.

If we assume that the supralinearity of LiF phosphor is due to the extra recombination centres produced by high energy particles, then the supralinearity should depend inversely on the linear energy transfer (LET) of the radiation. This agrees with the findings of other workers^{2,5}. This hypothesis can also explain why a phosphor is sensitized when annealed at 280° C for one hour after irradiation with 10⁵ rads gamma ray (⁶⁰Co) (ref. 6), simply because the lifetime of these centres at 280° C is much longer than one hour (the value of the 430° C peak by extrapolation is $T_{1/2} = 6$ hours). I hope to publish a fuller report of these experiments in due course.

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Relativistic Behaviour of Moving Terrestrial Clocks

EINSTEIN concluded in his 1905 paper on relativity that "a balance-clock at the equator must go more slowly, by a very small amount, than a precisely similar clock situated at one of the poles under otherwise identical conditions"¹. At that time, of course, he had not yet developed the general theory and did not realize that the difference in the gravitational potential at the equator and poles would just cancel the time dilatation from the difference in surface speeds, as has been pointed out recently by W. J. Cocke². Cocke has also shown that, in so far as the Earth assumes the shape of the geoid (and weak tidal fields can be neglected), its entire surface is an "equal time" surface. There remains an interesting question, however: What would be the rate of a standard clock that is moving

relative to stationary standard clocks on the geoid? The standard answer that moving clocks run slow by the well known factor $(1 - v^2/c^2)^{1/2}$ is almost certainly incorrect. The purpose of this note is to discuss this question of the rate of moving terrestrial clocks relative to stationary ones.

According to relativity theory, a clock records proper time. We are interested in finding the proper time for a body that is moving in a time-independent, permanent gravitational field. The notation and terminology of Møller³ will be used. According to Møller (Chapter VIII, equation 99), the rate of a moving standard clock in a gravitational field is given by

$$d\tau = dt(1 + 2\chi/c^2 - u^2/c^2)^{1/2}$$

where τ is the proper time, χ is the scalar gravitational potential, and u is the speed relative to a coordinate system in which t is the coordinate time. We are concerned with a weak gravitational field ($\chi \ll c^2$) and slow speeds ($u^2 \ll c^2$), so the approximation

$$d\tau \approx (1 + \chi/c^2 - u^2/2c^2)dt \quad (1)$$

will be adequate.

Let us consider a special case which is easy to visualize and can be generalized if desired. Consider an airborne clock at an altitude h directly above the equator and moving with a ground speed v , either directly East ($v > 0$) or directly West ($v < 0$). Relative to a non-rotating reference system, the speed of this clock is $u = v + (R+h)\Omega$, where Ω is the Earth's angular velocity and R is its radius. The scalar gravitational potential at the airborne clock is $\chi = -GM/(R+h)$, where G is the gravity constant and M is the Earth's mass. The speed of a ground based reference clock is $u_0 = R\Omega$, however, and its scalar potential is $\chi_0 = -GM/R$. Hence, applying equation 1 to the airborne clock gives

$$d\tau = [1 - GM/c^2 R(1 + h/R) - (R\Omega(1 + h/R) + v)^2/2c^2]dt$$

and to the ground based clock gives

$$d\tau_0 = [1 - GM/c^2 R - R^2\Omega^2/2c^2]dt$$

These equations give the time intervals of the respective clocks relative to an imaginary background system of coordinate clocks. But only proper time is recorded by real clocks; the coordinate time is not recorded. The unknown coordinate time can be eliminated, however, by taking the ratio $d\tau/d\tau_0$. Assuming $h \ll R$ and expanding to lowest order in all terms, a little algebra gives

$$d\tau/d\tau_0 = 1 + gh/c^2 - (2R\Omega v + v^2)/2c^2 \quad (2)$$

where $g = GM/R^2 - R\Omega^2$, the measured surface value of the acceleration of gravity at the equator. Equation (2) is remarkable in that it predicts that an airborne clock may run fast or slow relative to a ground based reference clock, depending on the sign of v . This directional dependence reflects the prediction of relativity theory that the relative rate of two clocks does not depend solely on the relative speed when both clocks experience acceleration. Moreover, aircraft ground speeds these days are great enough so that this directional dependence should be appreciable with modern, portable atomic clocks.

For example, suppose a jet plane carrying a portable atomic clock were to circumnavigate the globe once at the equator while flying at an altitude of 10 km (33,000 feet), with a ground speed of 300 m/s (670 miles/hour). Equation (2) predicts that, relative to an identical reference clock kept at the home port, the airborne clock would lose about 130 ns after an eastward circumnavigation, but would gain about 290 ns after a westward circumnavigation. These time offsets, particularly because they are positive for one direction and negative for the other, should be perceptible above the inherent drift of modern atomic clocks. The hydrogen maser clock is said to have an inherent drift of less than 1 part in 10^{14} (ref. 4). An experiment such as this one might empirically resolve once and for all the long-standing debate on the clock paradox⁵.

Finally, when time standards of the world's laboratories are intercompared by transporting portable standards between them⁶, any relativistic time corrections arising from interlaboratory transit would need to take into account the directional effects discussed here.

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BIOLOGICAL SCIENCES

Amino-acid Sequence of the Q β Coat Protein

THE RNA phages provide an excellent system in which to determine the primary structure of a messenger RNA and relate this to the proteins which it codes^{1,2}. By establishing the frame of reading, it is then possible to locate initiation and termination sites as well as extragenic regions^{3,4}. Our previous work concerned the amino-acid sequences of the f2 and R17 coat proteins^{5,6}. Using R17 it was possible to identify the initiation region corresponding to the coat protein cistron⁷; the termination region corresponding to the carboxy-terminus of the same cistron⁴; and an internal region of 57 nucleotides out of this cistron corresponding to residues 81-99 of the coat protein³.

The oligonucleotides, from R17 which have been sequenced were obtained by enzymatic fragmentation^{3,4}. With Q β , it proved possible to purify a specific replicase which could then be used to achieve partial synthesis of the RNA *in vitro*⁸. Analysis of the RNA pulse labelled *in vitro* has established the sequence of the first 175 nucleotides from the 5' end⁹. To relate the nucleotide and amino-acid sequences of Q β , we have analysed the structure of the Q β coat: the protein coded by Q β RNA that can be most readily obtained. Here we compare its sequence with that of the coat protein of the antigenically unrelated bacteriophage f2 and relate it to the nucleotide sequences already obtained from Q β RNA.

Stepwise Edman degradation performed on the coat protein revealed the sequence Ala-Lys-Leu-. One carboxy-terminal tyrosine residue (per molecular weight 14,000) was found by digestion with carboxypeptidase A. A tryptic digest of the performic acid oxidized protein was separated by ion exchange chromatography on 'AG 50-X4' resin and by gel filtration. The purity of the peptides was checked by paper electrophoresis and chromatography. Sequences in the smaller peptides were established by Edman degradation, digestion with leucine aminopeptidase and carboxypeptidase A and B⁹. The location of amides was in some instances inferred from enzymatic digestion and the electrophoretic mobilities¹⁰. Larger peptides were subjected to enzymatic fragmentation by chymotrypsin and subtilisin, and the resulting peptides were sequenced as described. The sum of the amino-acids from all tryptic peptides is in good agreement with the amino-acid composition of the Q β coat protein. Digestion

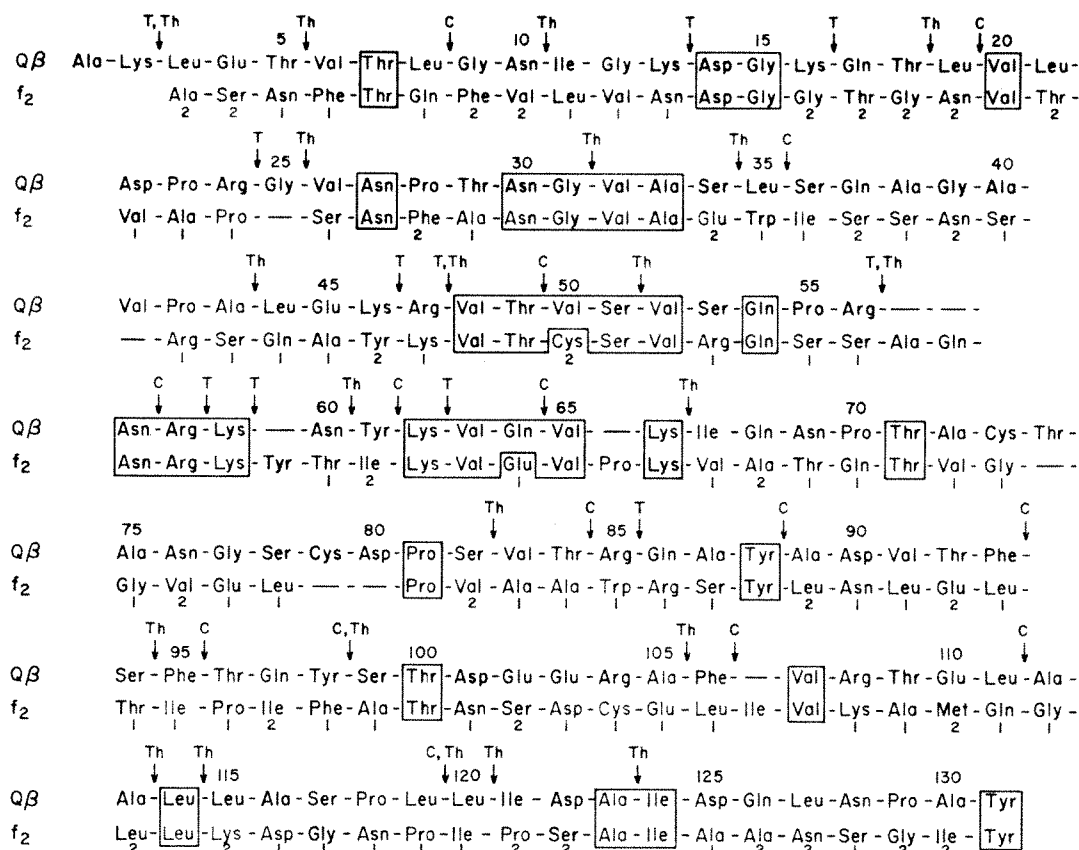


Fig. 1. Amino-acid sequence of Q β coat protein. The location of enzymatic splits used to establish the sequence is shown by arrows: T, trypsin; Th, thermolysin; C, chymotrypsin. The sequence of the f2 coat protein 5 has been provisionally aligned with that of the Q β protein to maximize the number of identical residues (see text). The numbers under each residue represent the minimum number of base changes per codon necessary to account for the amino-acid interchanges at each position in the f2 and Q β coat proteins.

of the intact protein by chymotrypsin, thermolysin and streptococcal proteinase gave overlap peptides which led to the arrangement of the tryptic peptides as shown in Fig. 1 and thus provided evidence for a linear structure for the Q β coat protein.

In the oligonucleotide fragment isolated from Q β RNA by Hindley and Staples¹¹, the five RNA triplets following the AUG codon code for amino-acids which are identical to those we have determined for the amino terminal sequence of the coat protein. Taken together with the finding of a peptide from an *in vitro* protein synthesizing system programmed by Q β RNA (unpublished results of M. Osborn, K. W. and H. Lodish) having the same amino-acid sequence but containing an amino-terminal formyl-methionine residue, these results establish that the RNA fragment of Hindley and Staples¹¹ contains the coat initiation region.

Knowledge of the coat protein sequence can confirm that the known 5'-terminal nucleotide sequence⁸ could not contain this initiation region. If the method of Billeter *et al.* can be extended to the nucleotide sequence beginning in the region 800-1600, where the coat protein cistron is expected⁸, it will be possible to compare directly the corresponding amino-acid and nucleotide sequences; otherwise, this comparison could be made by the methods successfully used in the case of R17 RNA³.

The coat protein of Q β bacteriophage is distinct from the corresponding f2 and R17 proteins in lacking the three amino-acids methionine, tryptophan and histidine. Like the f2 and R17 sequences^{5,6}, the Q β coat protein has intermittent stretches—up to twelve amino-acids—of non-polar residues. Although the Q β coat protein is only two residues longer than the f2 coat protein, it has eight more acidic and basic residues. In spite of these differ-

ences, however, the two proteins probably fold similarly to form the virus capsid. When the f2 and Q β sequences are aligned starting at the amino-terminus, sixteen out of 129 residues are identical and 46 (36 per cent) can be accounted for by single base changes. The percentage of residue interchanges that can be accommodated by alteration of a single base is within the range expected by comparison of random sequences¹². If the two proteins are aligned so that the carboxy-terminal residues coincide, eighteen of the residues are identical and 50 (39 per cent) can be accounted for by single base changes. A greater degree of homology is achieved, however, if insertions and deletions are allowed (Fig. 1). Thus insertions of Gly at position 25, Val at 41, Thr at 74, Cys at 79, Asp at 80, and deletions between 56-57, 59-60, 65-66 and 106-107 permit the alignment of residues 30-34, 48-52 (allowing for a Val for Cys replacement), 57-59 and 62-65 (allowing for a Gln to Glu substitution), using the Q β numbering system. In this way thirty residues occupy identical positions, and 62 (48 per cent) can be accounted for by single base changes. Although certain regions seem to be conserved, there is no evidence for any restriction in the rest of the protein. This, however, is only a provisional comparison, and other alignments might well allow a greater degree of homology.

The mutational events necessary to account for this situation are well accepted for DNA coded proteins, but they have not yet been established for phages with RNA as the genetic information. It may be that in aligning these coat protein sequences we are comparing the end products of a convergent evolutionary process and that the identities observed are fortuitous. Examination of a coat protein from the third class of RNA phages may clarify this matter.

Certain regions in both proteins that resemble each other chemically include the carboxy-terminal section from the residues 116–131 which is devoid of basic and aromatic residues, and the region from residues 42–65, in which three stretches of contiguous residues can be aligned so that the f2 and Q β sequences are identical. A similar correspondence is found between f2 and the antigenically distinguishable¹³ but related fr coat protein¹⁴. Although the f2 and Q β coat proteins both contain two cysteine residues with free SH groups, the cysteines occupy different positions in the two proteins. Functionally they are probably not important, for the coat protein from GR and SD (which comprise a third group of antigenically unrelated RNA phages¹⁵) is entirely devoid of cysteine. Clearly, a knowledge of invariant regions in the coat proteins would help in delineating the requirements for capsid formation and other functions in which the coat protein is presumably involved, such as regulation of the translation of the replicase cistron^{16,19}, stimulation of the rate of coat protein synthesis²⁰ and lysis of the host cell²¹. Sequence data on proteins in the cysteine deficient class must be obtained, however, before even tentative interpretation of structure-function or evolutionary relationships can have any validity.

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of this class in this strain. C57Bl/6 mice whose γG_{2a} molecules express the *Ig-1b* locus can produce antibodies reacting with *Ig-1a* determinants, if they are injected with 5563 myeloma protein in a suitably immunogenic form; but CBA mice cannot. The *Ig-1* locus is expressed on the constant region within the Fc fragment¹. It has been found that mice producing antibodies against these determinants also produce antibodies against idiotype—myeloma specific—determinants located within the Fab fragment². Mice immunized with non-conjugated myeloma protein either produce antibodies against both allotypic and idiotype specificities or they produce nothing against either determinant. Thus mice cannot detect idiotype determinants on non-conjugated myeloma protein with allotypic determinants that their own immunoglobulins possess. Cohn *et al.*³ have suggested that the Fc portion of the molecule acts as a “carrier” for the Fab portion, in the sense that an otherwise non-immunogenic determinant (the “hapten”) becomes immunogenic if attached to a structure which is itself immunogenic (the “carrier”). It has been shown that the “carrier effect” of Ovary and Benacerraf⁴ increases the immune response to the “hapten equivalent” by virtue of antigen-mediated cell cooperation^{5,6}.

To test the hypothesis that an antigenic determinant on the Fc acts as a carrier for determinants on the Fab, 2,4-dinitrophenol (DNP) was conjugated to 5563 myeloma protein and this alum-precipitated conjugate, with *Bordetella pertussis* as adjuvant, was injected into CBA mice. The unaltered Fc portion of the molecule could not act as the carrier for the reasons stated. But in these experimental conditions the 5563 protein has a DNP determinant to act as the “carrier”.

The immunological response to an antigenic determinant is increased if the host is presensitized to the carrier⁴. This presensitization engenders a population of helper cells, specific for the carrier, which are thought to be thymus-derived. Painting of the skin, with 1-fluoro-2,4-dinitrobenzene (FDNB), leads to cell-mediated immunity specific for DNP, which is almost certainly mediated by thymus-derived cells. Accordingly the mice were skin painted with FDNB to provide them with a population of thymus-derived, DNP-specific helper cells.

Protein from myeloma 5563 was extracted from the solid tumour by homogenization in 0.25 M sucrose containing 0.004 M MgCl₂ followed by centrifugation at 2,000g. The protein in the supernatant was further purified by precipitation in 50 per cent saturated ammonium sulphate and finally by ion exchange chromatography on DEAE-cellulose (Whatman DE-32) in 0.01 M Tris buffer (pH 8.1)–0.05 M NaCl. DNP-5563 conjugate was prepared by gently stirring the protein in 0.5 M sodium bicarbonate with FDNB for 1 h at room temperature, followed by dialysis. The molar ratio of DNP to 5563 protein, determined spectrophotometrically, was 3.2 : 1. Fab and Fc fragments were prepared by papain digestion followed by ion exchange chromatography.

CBA mice, 2–3 months old, were presensitized to DNP by painting their shaved abdomens with 50 μ l. of 0.5 per cent FDNB in a 1 : 1 mixture of olive oil and acetone. Ten days later the mice were injected with either 0, 25, 50, 75 or 100 μ g of alum precipitated DNP-5563 plus 2×10^9 formalin-killed *B. pertussis* organisms (kindly provided by Dr J. Cameron of Burroughs Wellcome Ltd). After three weeks, all the animals were boosted with 100 μ g of DNP-5563 in saline, and 10 days later the mice were bled. The anti-idiotypic antibody content of the individual sera was measured by means of a co-precipitation assay.

Each serum was tested at four dilutions. Ten μ l. of 10^{-8} I-5563 Fab (6×10^{-8} M) was added to 50 μ l. of antiserum diluted in 1 : 24 normal mouse serum diluted in borate buffer⁷. The antigen-serum mixture was incubated for 30 min at room temperature; then 50 μ l. of rabbit anti-mouse immunoglobulin serum was added to each tube,

Ability of CBA Mice to produce Anti-idiotypic Sera to 5563 Myeloma Protein

MICE can produce isoantibodies against immunoglobulins which carry allotypic determinants not present on their own immunoglobulin. For instance, the plasmacytoma 5563 (a γG_{2a} protein) arose spontaneously in C3H mice and expresses the *Ig-1a* locus like normal immunoglobulin

the contents of which were thoroughly mixed before being incubated for 30 min at 4° C. It is clear that the rabbit anti-mouse immunoglobulin serum cannot have any specificity for 5563 Fab. This was ensured by absorbing the serum with normal mouse immunoglobulin Fab made insoluble by conjugation to 'Sephadex', the absorption being repeated until no precipitation of ^{125}I -5563 Fab was obtainable. After incubation, the tubes were then centrifuged at 2,000g for 30 min. The supernatant was removed and the radioactivity of the precipitate measured in a scintillation counter. The \log_2 of the dilution of antisera that would bind 50 per cent of the radioactive 5563 Fab was calculated.

The anti-5563 idiotype antibody response was dependent on the amount of alum-precipitated antigen used (Fig. 1). In those animals which were boosted but had received no alum-precipitated antigen, little or no serum antibody could be detected. The animals which received the most alum-precipitated antigen produced the most antibody. The response between these two extremes was linear.

Another set of mice were treated as described, but they were not painted with FDNB. Instead, they were injected with alum-precipitated DNP-5563, plus *B. pertussis*, and were boosted with DNP-5563 in saline. There was no detectable anti-5563 Fab antibody in the sera of any of these mice.

Only 5563-Fab inhibits the binding of radioactive 5563-Fab by CBA anti-5563 (Fig. 2). The immunoglobulin, and immunoglobulin fragments which did not inhibit specific binding, included (1) immunoglobulins with the same class-specific and allotypic determinants—for example, 5563-Fc and LCP-1 protein; (2) immunoglobulins with different class and therefore different allotypic determinants—for instance, MCP-25 and MOPC-104G proteins; (3) immunoglobulins with the same L-chain type (K)—for instance, LPC-1 and MPC-25 proteins; and (4) an immunoglobulin with a different L-chain type (λ)—MOPC-104C protein. The constant region of all mouse L-chains of the same type tested so far has been shown to have the same amino-acid sequence. Furthermore, it has been shown that mouse myeloma proteins of the same class and allotype differ in their H-chain amino-acid sequence only in the N-terminal variable region. The antigenic determinants detected are therefore peculiar to 5563-Fab, and are therefore idiotype.

CBA mice, which do not in normal circumstances respond to an injection of alum-precipitated 5563 mye-

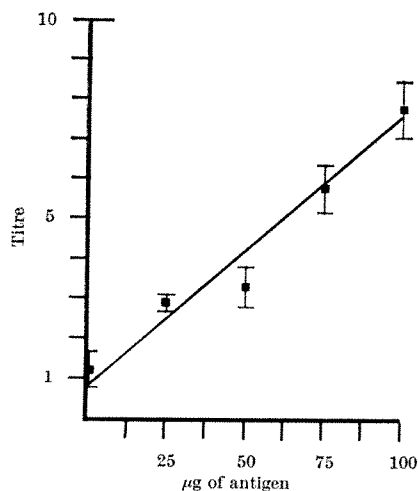


Fig. 1. Antigen dose response. The abscissa represents the amount of alum-precipitated DNP 5563 used to prime CBA mice which had been skin painted with 0.5 per cent FDNB 10 days previously. All mice received 100 µg of DNP 5563 in saline 3 weeks later. The ordinate represents the \log_2 of the dilution of antisera that would bind 50 per cent of the radioactive 5563 Fab at a final concentration of 10^{-8} M. Each point represents the mean of the individual values of six mice. The vertical bars represent the standard error for each group of six mice.

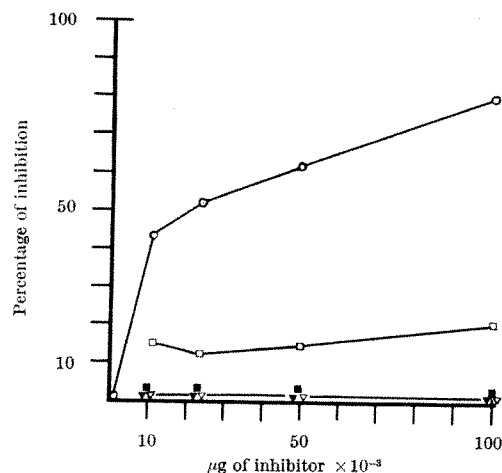


Fig. 2. Inhibition, by various myeloma immunoglobulins, of the binding between CBA anti-5563 and labelled 5563 Fab. Ten µl. of various concentrations of inhibitor was added to 50 µl. of a dilution of anti-serum that would bind 80-90 per cent of the radioactive 5563 Fab. After thorough mixing, 10 µl. of ^{125}I -5563-Fab (6×10^{-8} M) was added. The contents of the tube were mixed and then incubated for 30 min at 20° C, after which 50 µl. of rabbit anti-mouse immunoglobulin was added. Mixing was followed by incubation for 30 min at 4° C, and centrifugation at 2,000g for 30 min, removal of the supernatant and measurement of the radioactivity in the precipitate. The degree of inhibition was calculated as a percentage of the amount of label precipitated in controls without inhibitor. The various purified myeloma immunoglobulins tested are: 5563 Fab, \square ; 5563 Fc, \circ ; LCP-1 (γG_{2a} Ig-1a), \blacktriangledown ; MCP-25 (γG_1 Ig-4a), ∇ ; MOPC-104 (γM), \blacksquare .

loma protein plus *B. pertussis*, could be made to do so by adding a DNP determinant to the myeloma protein, and presensitizing the mice to DNP. These mice must have possessed antibody forming cells and their precursors with receptors for this idiotype determinant. All that seems to be required for the response was that the immunogen should be presented in an appropriate way to these cells. The observation that the animals have to be presensitized to this new determinant supports the argument that the conjugation has not non-specifically increased the immunogenicity of the 5563 molecule. The activating effect of skin painting with FDNB supports the hypothesis that the DNP group is acting in the role of the "carrier", as proposed by Mitchison and Rajewsky^{5,6} (see also ref. 8). According to this hypothesis the DNP-specific helper cells present the idiotype determinant to antibody forming cell precursors.

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Immunological Paralysis induced by an Idiotype Antigen

A DIRECT association presumably exists between idiotype specificity and the structure of the antibody site. The level of a particular idiotype should therefore vary in normal individuals and could reach relatively high levels after prolonged immunization with a homogeneous

antigen. There must therefore be a mechanism which prevents autoimmunization by idiotypes. Two such mechanisms which have been suggested are (1) that an animal is tolerant of (paralysed by) its own idiotype determinants¹, and (2) that an antigen can only be an immunogen when it carries two widely separated determinants which enable it to participate by means of a carrier-effect mechanism²⁻⁴, in a cooperative act between two kinds of cell involved in the immune response⁵. Hypothesis (2) is contradicted by the results described in the previous article⁶, where the dinitrophenyl derivative of myeloma protein 5563 (DNP-5563) failed to elicit an immune response without previous or concomitant immunization against the DNP group, and also by the known capacity of multideterminant antigens such as mammalian γ -globulins to induce paralysis rather than immunity in adult animals.

Extrapolation from experiments with bovine (BGG) and other γ -globulins in CBA mice (see ref. 7) suggests that 2 μ g of 5563-myeloma protein would be insufficient to induce a state of immunological paralysis, whereas 100 μ g or more would be enough. The experiments with BGG showed that the state of paralysis was acquired by a CBA mouse progressively over a period of up to 3 weeks after the injection of the paralytogen. Unpublished findings of G. M. I. have shown that the level of 5563-idiotype (expressed in terms of γ -globulin bearing this determinant) in four inbred strains of mice is about 35 ng/ml. of serum. We have concluded from this observation that a state of immunological paralysis is unlikely to exist for the 5563-idiotype. The observation of several workers that various anti-idiotype antibodies can be raised experimentally (see ref. 6) strongly supports this view. Consequently we decided to find out if 5563-myeloma protein was capable of inducing a state of immunological paralysis to its idiotype determinant in adult CBA mice.

Male CBA mice, aged 3-5 months, were used. Myeloma protein 5563 was prepared from solid tumour as described⁶. Purified 5563-protein which was to be tested for its properties as a paralytogen was dialysed against physiological saline and then centrifuged at 30,000*g* before being injected intraperitoneally. The half-life of 5563-protein in the blood of CBA mice was measured as described pre-

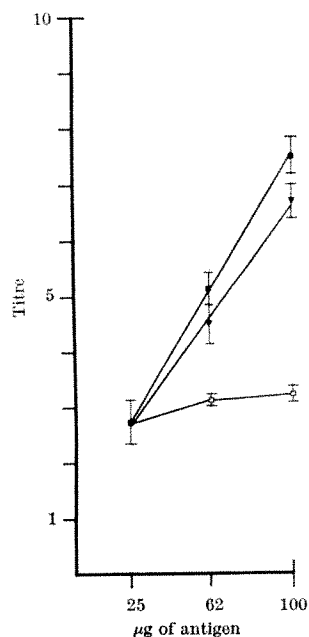


Fig. 1. The anti-5563 idiotypic response in CBA mice challenged with different amounts of antigen. Each point represents the mean titre (ref. 6) in six experimental animals. ■, Controls; ▼, mice injected with 0.5 μ g particle free 5563-protein 4 h before challenge; □, mice injected with 100 μ g of 5563-protein 28 days before challenge. Vertical bars represent one standard deviation.

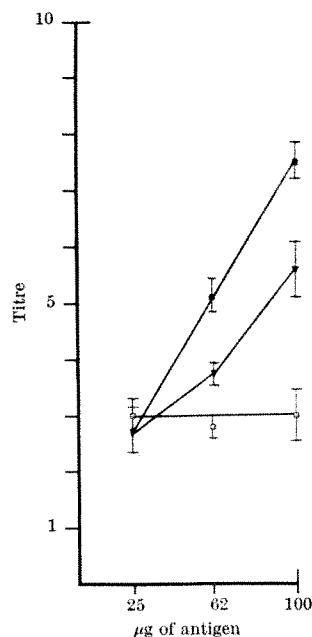


Fig. 2. Similar to Fig. 1 except that mice were injected with 1.4 μ g of 5563-protein 4 h before challenge (▼) and with 320 μ g of 5563-protein 28 days before challenge (□).

viously⁸, using ¹³¹I-labelled protein prepared by the ICI method of McFarlane⁹. Experimental and control mice were tested for their ability to make anti-5563 myeloma-specific antibodies (anti-idiotype) by the method of Iverson⁶.

A group of CBA mice were injected intraperitoneally with 100 μ g of particle-free 5563-protein (another group received 320 μ g). Twenty-eight days later all these mice, together with controls, were skin painted with 0.05 ml. of a 1:1 mixture of acetone and olive oil containing 0.5 per cent fluorodinitrobenzene (FDNB). After a further 10 days sub-groups from each major group of mice were challenged with 25, 62 or 100 μ g of alum-precipitated DNP-5563-protein conjugate (molar ratio, 3.2:1) together with 20×10^8 *Bordetella pertussis* organisms. The challenging doses used were in a range where it had previously been shown that there was a distinct dose-response relationship between the dose of antigen (idiotype) and the antibody response. Ten days after the challenge, all the mice were boosted by an intraperitoneal injection of 100 μ g of 5563-protein in saline. After a further 10 days the mice were bled individually and the individual sera titrated for their content of anti-idiotype antibody using a co-precipitation assay as described⁶.

One mg of ¹³¹I-5563-protein was injected into five CBA mice, and an estimate was made of the half-life of the protein in the blood: this was 5.4 days. From this rate we calculated that mice injected with 100 μ g and 320 μ g of 5563-protein would have in their extracellular body fluids a total of 0.5 μ g and 1.4 μ g of 5563-protein, respectively, at the time of challenge. This small amount of antigen might interfere with the challenging injection, so we included groups of control mice which were injected with these amounts of 5563-protein 4 h before challenge.

The results (Figs. 1 and 2) show (1) that the amounts of anti-idiotype antibody produced in response to the different challenging doses of antigen correspond to the amounts obtained previously; (2) that 1.4 μ g of 5563-protein injected as a particle-free solution 4 h before challenge had a small effect in lowering the ensuing anti-idiotype response; (3) that 100 μ g or 320 μ g of particle-free 5563-protein, injected a few weeks before challenge, results in a complete suppression of the anti-idiotype response.

If, as might be expected, the idiotypic antigens of normal antibodies behave like the myeloma protein

described here, then the function of paralysis by such antigens may have an important biological function. For example, a single species of antibody—that is, a population of antibody molecules homogeneous in both specificity and affinity—will normally be at concentrations too low for its idiotype to have induced either immunity or paralysis. If, however, the animal is immunized with the appropriate antigen the concentration of idiotype may rise to concentrations where either paralysis or immunity could be induced. It is known that the injection of sufficient amounts of antibody-antigen complex can lead to the production of anti-idiotypic antibody¹. In a situation where such complexes may form naturally it is clearly important that the relevant idiotypic determinants should have been able to induce paralysis readily, if autoimmunity is to be avoided. Conservation of primitive features among the γ -globulins of different mammalian species¹⁰, together with a strong selective pressure favouring the evolution of a mechanism whereby γ -globulin molecules, although antigenic, are non-immunogenic, may offer an explanation of the immunological situation such as that which follows the injection of BGG into mice.

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Two Anti-inflammatory Components in Antilymphocytic Serum

ANTILYMPHOCYTIC serum (ALS) is widely known for its ability to impair immunological responsiveness, especially of cell-mediated immunity^{1,2}. The main immunosuppressive component of ALS *in vivo* is IgG (7S- γ) immunoglobulin^{3,4}. Administration of ALS to animals also causes a marked reduction in inflammation, as judged by various experimental models⁵⁻⁷, without causing increased adrenal activity^{5,6}.

In this study anti-guinea-pig lymphocytic serum, as well as anti-rat lymphocytic serum, was shown to have anti-inflammatory activity in the rat. An attempt was therefore made to separate anti-inflammatory components from the immunosuppressive component (IgG, 7S- γ) of antilymphocytic sera using gel-filtration chromatography.

Anti-rat (Wistar strain) lymphocytic serum (ARLS) and anti-guinea-pig (Hartley strain) lymphocytic serum (AGPLS) were raised² separately in New Zealand white rabbits (2–3 kg body weight) by giving two intravenous injections, 14 days apart, of a suspension of 10^9 viable thymocytes in Hanks balanced salt solution. The rabbits were bled 7, 10 and 14 days after the final injection of thymocytes. Complement in the pooled serum was inactivated (heated at 56° C for 30 min), and erythrocyte cytotoxic antibodies were absorbed by addition of 15 per cent packed homologous erythrocytes (37° C for 30 min). The antisera, and normal rabbit serum (NRS) for use as a control, were freeze-dried and stored at –20° C. Before use, freeze-dried products were reconstituted with either sterile distilled water or isotonic saline. ARLS and AGPLS were fractionated as shown in Fig. 1 and their

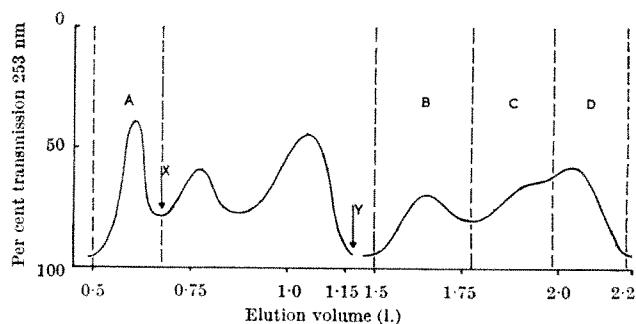


Fig. 1. Fractionation of the ALS was performed on a column of 'Sephadex G-150' (5 × 100 cm) eluted by upward flow at 50 ml/h with 0.5 M NaCl. Samples of 1.5 g dry weight of the sera (ARLS or AGPLS) were subjected to recycling chromatography using automatic protein fractionation and ultraviolet analysis equipment (LKB). Recycling was started at X and terminated at Y at which stage eluant collection began. The fractions taken for anti-inflammatory testing were: A, macroglobulin fraction (19S); B, IgG (7S- γ) fraction; C, AIF fraction; D, albumin fraction.

immunosuppressive potency was assessed against the skin homograft reaction.

Female Wistar rats (approximately 100–120 g) were recipients for full-thickness tail skin homografts taken from hooded rat donors (groups of eight rats). The graft area on a flank was covered with 'Vaseline'-gauze ('Jelonet', Smith and Nephew) and protected with a light casing of self-adhesive bandage ('Prestoband', Vernon) for 6 days.

The anti-inflammatory activity of both ARLS and AGPLS, or of their chromatographically separated fractions, was determined using carrageenin induced oedema of the rat hind foot^{8,9}. Groups of five Wistar rats (males, 140–180 g) were used for assays. Oedematous inflammation was produced by injection of 0.1 ml. of 1.0 per cent carrageenin (Marine Colloids) in isotonic saline into the plantar aponeurosis of the right hind foot. The ALS material (or NRS) was injected subcutaneously into the interscapular region at the time of injection of carrageenin into the foot. The volume of the foot was measured plethysmographically⁹ before injection of carrageenin and at the height of the inflammatory reaction, 5 h later, anti-inflammatory activity was calculated as the percentage inhibition of paw swelling compared with control groups.

Table 1. EFFECT OF ALS AND NRS ON CARRAGEENIN-INDUCED OEDEMA OF THE RAT HIND FOOT

Treatment (subcutaneous)*	Dose (mg dry wt/kg body wt)	Anti-inflammatory effect (percentage inhibition of oedema formation)	Significance (P) compared with saline treated control
NRS	1,000†	17	N.S.
AGPLS	1,000	48	< 0.001
ARLS	1,000	68	< 0.001

* Freeze-dried materials were reconstituted before injection—control animals received an equivalent volume of sterile isotonic saline.

† 1,000 mg of freeze dried rabbit serum is approximately equivalent to 12.5 ml.

Both ARLS and AGPLS significantly suppressed oedema (Table 1), though ARLS had the greater efficacy. Table 2 shows a representative result for the anti-inflammatory effect of various ALS fractions obtained by 'Sephadex' chromatography (Fig. 1). Whereas ARLS possessed anti-inflammatory activity in the IgG (7S- γ) fraction, AGPLS IgG (7S- γ) did not produce a significant reduction of inflammation in the rat. In both antisera, however, significant additional anti-inflammatory activity was found in the fraction called AIF (non-specific anti-inflammatory factor). These findings may account for the fact that AGPLS had previously been found less effective than ARLS in suppressing inflammation in the rat (Table 1). Because the AIF fraction from both ARLS and AGPLS had anti-inflammatory activity in rats, we concluded that there are two anti-inflammatory components in ALS, namely, IgG (7S- γ) and AIF, but only the former had species specificity. The AIF was probably produced in the animal in which the antisera was raised as a conse-

Table 2. EFFECT OF 'SEPHADEN'-SEPARATED FRACTIONS* OF ALS ON CARRAGEENIN-INDUCED OEDEMA OF THE RAT HIND FOOT

Treatment (subcutaneous)	Dose† (mg dry wt/kg body wt)	Anti-inflammatory effect (percentage inhibition of oedema formation)	Significance (P) compared with saline treated control
ARLS fraction*			
A	150	17	N.S.
B	160	53	< 0.001
C	200	48	< 0.001
D	220	17	< 0.02
AGPLS fraction*			
A	140	13	N.S.
B	170	15	N.S.
C	200	48	< 0.001
D	210	15	N.S.

* See Fig. 1 for elution diagram and explanation of A, B, C, D.

† The dose of chromatographic fractions was equivalent to the original dose (1,000 mg/kg) of whole serum.

Table 3. EFFECT OF 'SEPHADEN' SEPARATED FRACTIONS OF ARLS ON SKIN HOMOGRAFT SURVIVAL IN RATS

Treatment	Dose*	Graft survival (days ± s.d.)
IgG (7S-γ)	200 mg/kg	20.8 ± 4.1
AIF fraction	250 mg/kg	10.7 ± 1.5
Controls	3.5 ml. saline	10.6 ± 1.4

* Administered subcutaneously on days 2 and 5 after grafting (hooded rat recipients; August rat donors of tail-skin grafts). Groups of eight rats were used for assay.

quence of the inflammatory—rather than the immunological humoral—response to foreign lymphocytes.

The results (Table 3) confirm that the IgG (7S-γ) fraction of ARLS is immunosuppressive in that it significantly delays rejection of rat skin homografts, a property not shared by the AIF fraction. It is well known that ALS administration delays or prevents rejection of homografts only in the species of animal against whose lymphocytes the antiserum was raised¹⁰.

Previous experiments have demonstrated the production, during acute inflammation, of an anti-inflammatory protein by a number of mammalian species (man¹¹, rat⁹ and guinea-pig¹²). This anti-inflammatory protein occurs in inflammatory exudate and to a lesser degree in serum, and is probably the same as the AIF of antilymphocytic sera, for it has similar elution characteristics on 'Sephadex' chromatography of cell-free inflammatory exudates^{9,12}. Unlike ALS IgG (7S-γ), the anti-inflammatory protein is not lymphocytotoxic (unpublished finding).

The physiological significance of the anti-inflammatory protein is unknown, but it might play a part in the homeostatic control of inflammation before resolution and repair at the inflammatory site.

Because only certain batches of ALS have anti-inflammatory activity—though still possessing immunosuppressive potency⁷—AIF is either variably produced during the raising of ALS and/or is not present at the particular time of collection of serum. The use of Freund's adjuvant in raising ALS should enhance the formation of AIF, for irritant substances implanted or injected into animals elicit formation of the anti-inflammatory protein^{9,11-13}.

ALS has aroused much interest in clinical medicine as the most powerful immunosuppressive agent known, and it is often chosen for the treatment of rejection crises of foreign organ transplants. It seems likely that transplant patients also produce the anti-inflammatory protein as a consequence of surgical trauma, and introduction of foreign tissues including ALS IgG (7S-γ). Surgery itself, for example, partial gastrectomy¹⁴, results in the formation of the anti-inflammatory protein. It is not yet clear whether the anti-inflammatory substances in ALS potentiate the immunosuppressive activity of ALS IgG (7S-γ). M. E. J. B. is in receipt of an MRC research grant.

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Invertebrate Protein Simulating Mediators of Delayed Hypersensitivity

THE aggregation of phagocytic cells at inflammatory sites is a characteristic feature of the defence of multicellular organisms against potentially injurious agents¹⁻⁴. From the point of view of comparative immunity, it is the one clear-cut similarity between vertebrates and invertebrates. Although multiple mechanisms may exist which control the accumulation of these cells, the obvious biological importance of this common defence system suggests the conservation of its principal elements in evolution. We have investigated the effects of macrophage-active factors from an invertebrate source on vertebrate (mammalian) inflammatory mechanisms.

Echinoderm phagocytes (coelomocytes) aggregate *in vivo* after an injection of inorganic particulate material, bacteria, or foreign cells⁵⁻⁷. Recently, Bang and Lemma have described the response of the sea star to a local injection of coelomocyte lysate into the coelomic cavity of the donor or other intact stars⁸. This response could be observed by direct microscopy of the living animal, and was characterized first by local, and later more peripheral clumping of coelomocytes within the respiratory papulae of the injected arm. We chose this whole cell lysate as the starting material for our investigation.

Sea stars (*Asterias forbesi*) were collected from the waters surrounding Woods Hole, Massachusetts, and "bled" by amputation of the tip of one or more arms; the coelomic fluid was drained into glass beakers. Following spontaneous agglutination and settling at 4° C, the coelomocytes were washed twice in seawater and a 20 per cent suspension (by volume) was lysed by brief ultrasonication. The product was centrifuged for 2 h at 60,000g and the clear supernatant concentrated by vacuum pressure dialysis to a final protein concentration of 3 to 5 mg/ml. The injection of as little as 20 μg of this supernatant in 0.1 ml. artificial seawater diluent into the arm of intact stars caused prompt, tight aggregation of coelomocytes circulating in the respiratory papulae of the injected arm. Aggregates of cells fixed to the walls of these structures and remained immobile for approximately 20 to 40 min, after which they slowly dispersed. This behaviour is essentially identical to that described previously⁸.

The activity of this material in mammalian systems was first investigated by intracutaneous injection of comparable amounts of whole coelomocyte lysate made isotonic by dialysis against 0.01 M phosphate-buffered saline (pH 7.4) into normal Hartley strain guinea-pigs. Begin-

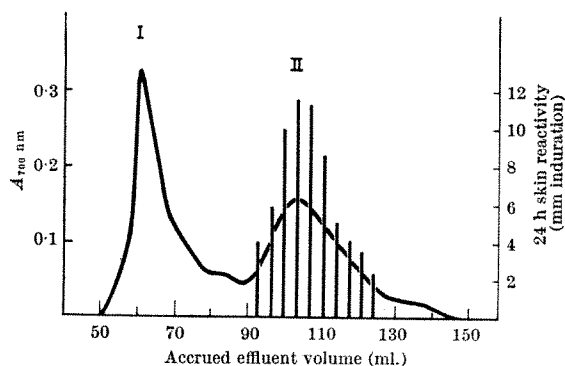


Fig. 1. Sea-star coelomocyte euglobulin preparation eluted from 'G-75' column by 0.01 M sodium acetate buffer in 0.15 M NaCl (pH 5.0), giving two major protein peaks revealed by Folin analysis. The vertical bars through peak II represent the diameters of 24 h indurated skin lesions produced by intracutaneous injection of 0.1 ml. of individual fractions eluted under this peak.

ning at 6 to 8 h, an erythematous indurated lesion resulted which reached its maximum size between 24 and 48 h, after which it slowly resolved. The response was temporally, physically and histologically indistinguishable from that of cutaneous delayed tuberculin hypersensitivity. Similar reactions could be elicited in normal rabbits, mice, sheep, rats and day-old guinea-pigs following injection of this material. In no instance was there evidence of immediate skin reactivity.

Initial characterization of the whole coelomocyte lysate by 'Pevikon' block electrophoresis in 0.05 M veronal buffer (pH 8.2) localized the skin reactivity to cathodically migrating proteins. Immunoelectrophoresis using hyper-immune rabbit serum against whole coelomocyte lysate demonstrated two proteins in this peak: a strong arc in the γ^1 position and a fainter arc more cathodic in migration. These proteins were concentrated in the euglobulin fraction precipitated by dialysis of whole lysate against large volumes of 0.005 M phosphate buffer (pH 5.5).

Chromatography on 'Sephadex G-75' of the euglobulin preparation of coelomocyte lysate resulted in the elution profile of Fig. 1. Skin reactivity was confined to the second of the two major peaks (peak II) with an elution volume of 105 ml. Previous calibration of this column with protein markers of known molecular weight indicated that this elution volume lay between that of ovalbumin (94 ml.) and chymotrypsinogen (111 ml.), corresponding to a molecular weight of 32,000 by the method of Andrews⁹. Pooling all fractions of peak II followed by further concentration resulted in a single arc on immunoelectrophoresis identical to the γ^1 arc seen in whole lysate (Fig. 2). Skin reactivity per μ g protein of this purified fraction was greatly enhanced compared with whole lysate, so that 1 μ g was sufficient to induce an indurated lesion 12 mm in diameter which persisted for 48 h. Skin tests with 20 μ g of pooled peak I protein were negative. From these experiments we conclude that the delayed skin lesion in normal animals is dependent on the presence of γ^1 protein in the test material, and its severity directly proportional to the concentration of this component.

Delayed hypersensitivity in mammalian species has been shown to parallel the inhibition of macrophage migration during short-term tissue culture when cell populations containing sensitized lymphocytes are exposed to homologous antigen *in vitro*¹⁰. The interaction of lymphocyte and antigen results in the release of a non-specific migration inhibitory factor (MIF) which presumably acts on the macrophage cell population to inhibit normal migration^{11,12}. In similar *in vitro* experiments normal guinea-pig peritoneal macrophages were exposed to sea-star coelomocyte euglobulin preparations and fractions eluted by 'G-75' column chromatography. Significant inhibition of macrophage migration was seen in those cultures containing the γ^1 protein. Migration in

Table 1. MIGRATION OF GUINEA-PIG PERITONEAL EXUDATE CELLS IN MEDIUM CONTAINING DIFFERENT FRACTIONS OF WHOLE SEA-STAR COELOMOCYTE LYSATE

Nature and concentration of material added to normal medium			
Euglobulin from whole lysate (20 μ g/ml.)	'G-75' peak I (20 μ g/ml.)	'G-75' peak II (10 μ g/ml.)	Euglobulin heated 56° C for 30 min (20 μ g/ml.)
64	126	53	88
41	107	54	115
60	173	43	120
39	109	67	100
32	61	53	99
81	185	44	81
55	100	55	
	36	39	
Mean: (53)	(112)	(51)	(100)

The values represent the area covered by migration expressed as the per cent of an equal number of control cultures run simultaneously in normal medium.

control cultures containing medium alone and in those containing 20 μ g/ml. 'G-75' peak I was similar. Medium containing 20 μ g/ml. euglobulin or 10 μ g/ml. 'G-75' peak II inhibited the average migration of macrophages to 51 and 53 per cent of control values respectively (Table 1). Inhibition of migration to the same degree could be demonstrated using macrophages separated from other cellular elements of the peritoneal exudate by plating on glass^{11,12}. Participation of the lymphocyte population in the exudate is not therefore required for the inhibition imposed by the presence of active sea-star material. Previous exposure and sensitization to cross-reacting antigens are therefore unlikely as an explanation of the action of this protein, for sensitized lymphocytes would be required for the generation of MIF. Peritoneal exudate cells exposed to medium containing 10 μ g/ml. 'G-75' peak II for 30 min and then washed and cultured in medium alone migrated to the same extent as controls, indicating that a primary toxic effect on the macrophages was unlikely. Exposure of the euglobulin preparation to 56° C for 30 min completely destroyed its ability to inhibit macrophage migration. From these experiments we conclude that inhibition of normal guinea-pig macrophage migration by sea-star coelomocyte lysate depends on the presence in the medium of the same protein as that responsible for the skin lesion in normal animals.

Intracellular proteins of the phagocytes of invertebrates may prove a fruitful source of evolutionary progenitors of those molecules concerned with immunological defence functions in the vertebrate species, as postulated by Burnet¹³. The position of the echinodermata immediately below the chordata in the phylogenetic scheme makes this large group of animals especially attractive as a starting point for such investigations. The possible relationship between the protein obtained from the sea-star coelomocyte and that produced by sensitized mammalian lymphocytes in the presence of homologous antigen is of particular interest. If the receptor sites on mammalian macrophages for the action of MIF are closely related or identical to that affected by the sea-star protein, these two effector molecules from different phylogenetic sources may be similar in structure as well as activity.

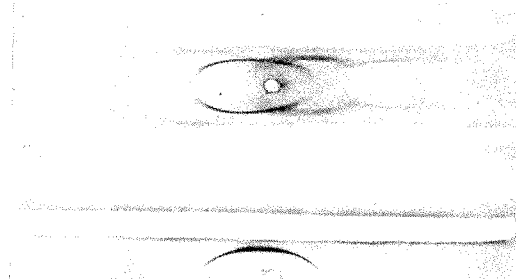


Fig. 2. Immunoelectrophoresis of coelomocyte lysate preparations; rabbit anti-whole coelomocyte lysate used in all troughs. Anode is to the right. Top: whole coelomocyte lysate; bottom: pooled fractions under peak II, 'G-75' column chromatograph of Fig. 1.

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Selective Suppression of Cell Mediated Immunity by Equine Anti-rabbit Lymphocyte Serum

WE have investigated the effect of antilymphocyte serum (ALS) on the humoral antibody response, in rabbits, which readily produce large amounts of circulating antibody to well defined antigens. ALS was made by injecting a horse intramuscularly with a mixture of 2×10^8 thymus and lymph node cells from Himalayan rabbits, mixed with incomplete Freund's adjuvant. Seven days later the same

place by sutures. The dressings were removed on day 5, and the grafts were considered rejected when the skin became hard and scabbed over.

In the first experiment (Table 1A) the rabbits received a 12 day intermittent course of ALS or NES. ALS produced a striking prolongation of skin graft survival, with a mean for the group of 17.8 ± 0.8 s.e.m. days, compared with 9.5 ± 1.2 s.e.m. days for the controls. By contrast, ALS had no effect on the level of antibodies to bovine serum albumin (BSA) as measured by the indirect haemagglutination test, using the microtitre technique¹ after coupling the BSA to the sheep erythrocytes by the bis-diazotized benzidine method². All rabbits treated with ALS made a normal primary response, and after another immunization a normal secondary response. All treated animals also had substantial quantities of antibodies against equine γ -globulin (EGG).

These findings were checked by giving two groups of white NZ rabbits skin grafts, as before, on day 0, but no ALS. They rejected the grafts as expected. They also made a normal primary and secondary response to BSA. The 12 day intermittent course of ALS or NES was given, starting on day 68, and a second skin graft from the same donor strain was made on day 70. The NES group rejected the grafts with a mean of 8.25 ± 0.4 s.e.m. days, but with the group treated with ALS the survival of grafts extended on average to 16.5 ± 1.2 s.e.m. days (Table 1B). Immunization was carried out on days 70 and 91 with ovalbumin (OA), which has no groups to cross-react with BSA or equine albumin. As can be seen from Table 1B, the group treated with ALS made more antibody to OA and to EGG than the group treated with NES.

Clearly in the rabbit, ALS selectively suppressed cell mediated immunity but not the production of humoral antibody, either to well defined antigens such as BSA or OA, or to antibodies against horse proteins in ALS. Although ALS is known to be ineffective in suppressing antibodies against itself³⁻⁵ and to act "more effectively on the cellular than the humoral response" in mice⁶, our results are significant in showing that this dichotomy of effectiveness is complete in rabbits.

Table 1. EFFECT OF ALS ON SKIN ALLOGRAFT SURVIVAL TIMES AND ON HUMORAL ANTIBODY PRODUCTION

Treatment	No.	Survival of skin graft (days \pm s.e.m.)*	Circulating antibody, mean $-\log 2$ haem. titre \pm s.e.m.						
			BSA † (days)			EGG (days)			
			0	14	21	28	0	21	28
(A)									
		First graft, day 0							
NES‡	8	9.5 \pm 1.2	0	4.1 \pm 0.8	6.0 \pm 0.6	10.7 \pm 0.7	0	10.0 \pm 0.7	10.0 \pm 0.9
ALS‡	8	17.8 \pm 0.8	0	5.0 \pm 1.2	6.4 \pm 0.6	12.6 \pm 1.2	0	11.0 \pm 0.4	10.0 \pm 0.4
(B)									
		Second graft, day 70							
			70	Ovalbumin ‡ days			70	91	98
NES¶	15	8.25 \pm 0.4	0	3.9 \pm 0.4		11.3 \pm 0.8	0	9.4 \pm 0.4	9.0 \pm 0.5
ALS¶	6	16.5 \pm 1.2	0	6.7 \pm 0.8		16.0 \pm 1.3	0	12.8 \pm 0.8	13.5 \pm 1.0

* Donors: black NZ male rabbits; recipients: white NZ male rabbits.

† Immunization on days 0 and 21 with 100 mg of BSA given intravenously.

‡ Immunization on days 70 and 91 with 100 mg of ovalbumin given intravenously.

§ 3 ml/kg given subcutaneously on days -1, -2, 0, 2, 4, 6, 8 and 10.

¶ 3 ml/kg given subcutaneously on days 68, 69, 70, 72, 74, 76, 78 and 80.

number of cells, this time from white New Zealand (NZ) rabbits were given, followed after 14 days by a similar immunization. The horse was bled a week later. The same horse had also been simultaneously immunized with mouse lymphoid cells and the antimouse component was active as an immunosuppressant.

The cytotoxicity titre of the anti-rabbit component was 1:27 against rabbit thymocytes and 1:243 against lymph node cells. The ALS was heated to 56°C to destroy complement and sterilized by passing it through a Seitz filter. The normal equine serum (NES) used in the control group was handled in the same way.

The day of grafting or immunization was designated day 0, and so the preceding days were negative and succeeding days positive. Full thickness pinch ear grafts were taken from black NZ donors and placed in beds on the dorsum of the ear of white NZ recipients, and held in

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Detection of Actomyosin-type Protein at the Surface of Dissociated Embryonic Chick Cells

WE have previously shown¹ that antibodies prepared against smooth muscle actomyosin and capable of reacting with fibroblasts of connective tissue and with thrombocytes (platelets) markedly reduced the aggregative competence of embryonic chick muscle and liver cells. These antibodies were type specific and did not react with skeletal or cardiac striated muscle actomyosin nor with the (Na⁺,K⁺,Mg²⁺)-ATPase from human erythrocyte ghosts². The same antibodies blocked the activity of the Ca²⁺-dependent myosin ATPase of smooth muscle actomyosin³. Antibodies directed against striated muscle actomyosin and capable of blocking the activity of the Ca²⁺-dependent ATPase of this protein did not produce the aggregation-inhibitory effect. This led to the suggestion that the effect was the result of the antibodies against smooth muscle actomyosin reacting with an actomyosin of similar type at the surface of the muscle and liver cells⁴.

These findings are in keeping with the theory that a surface-localized actomyosin-like protein with rudimentary contractile properties is involved in cell adhesion⁵ and might be concerned with the movement of linkage sites⁶⁻⁷, on which adhesion may depend. But the results hitherto did not provide evidence indicating attachment of the "inhibitory" antibodies to analogous antigens at the cell surface: such evidence would validate the suggestion that the aggregation-inhibitory effect was accompanied by the reaction of the "inhibitory" antibodies with surface-localized antigens. We now report the results of experiments, using the fluorescent antibody technique, which reveal the presence of actomyosin-like protein with an antigenic similarity to smooth muscle actomyosin at the surfaces of muscle and liver cells from chick embryos.

The required tissue from 9 day old chick embryos was dissociated into separate cells with 0.25 per cent trypsin in Hanks balanced salts solution⁸. The cells were suspended in Eagle's minimal essential medium containing 10 per cent calf serum, and cultured in Leighton tubes at 37° C for 24 h. The γ -globulin fractions prepared from antisera against smooth muscle actomyosin of chicken gizzard (GAM) and chicken pectoralis muscle actomyosin (PAM), respectively, were conjugated with fluorescein isothiocyanate^{9,10}. The cells attached to the coverslip were washed with Dulbecco physiological saline before being treated with the conjugate¹¹. The preparations were washed thoroughly and mounted in 70 per cent glycerol-glycine buffer (pH 8.6)¹⁰.

The surface of the cells from both the embryonic muscle and liver tissue reacted—according to the distinctive green fluorescent staining—only with the anti-GAM γ -globulins. These, we have shown (unpublished results of R. B. K., B. M. J. and U. G.-S.), inhibit cell aggregation as effectively as antiserum against actomyosin from uterus smooth muscle (anti-UAM)⁴. The anti-GAM γ -globulins were irreversibly attached to surface antigenic sites. The conjugated γ -globulin fractions from both the pre-immunization rabbit serum (control) and the anti-PAM serum did not stain the cell surface.

Thus fluorescent anti-GAM γ -globulins can react with surface-localized actomyosin-like protein having antigenic properties similar to that of smooth muscle actomyosin. Such a reaction between GAM γ -globulins and the surface of the embryonic chick cells, leading to a reduction in the aggregative

competence of these cells, strengthens the view¹ that cell adhesion involves the participation of surface-localized actomyosin-like protein.

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Structure of the Subunits in the Thick Luminal Membrane of Rat Urinary Bladder

A SUBSTRUCTURE of hexagonally arranged subunits has been reported in the luminal membrane of the superficial cells lining the rat urinary bladder^{1,2}. Preliminary examination of electron micrographs of negatively stained membranes in an optical diffractometer (unpublished observations of R. M. H., R. W. Horne and G. J. Hills) suggested that each subunit was a hexamer composed of six smaller particles. Vergara, Langley and Robertson³ described a similar hexagonal lattice in the mouse bladder luminal membrane. Their optical filtering technique revealed a lattice of hexamers skewed by 19° to the axis of the lattice. This communication describes how further detail has been resolved in the substructure of the membrane by a modification of the linear integration technique of Markham, Hitchborn, Hills and Frey⁴.

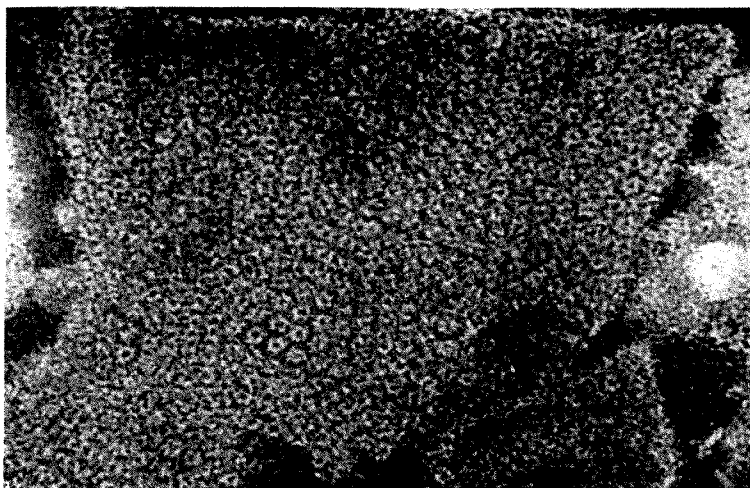


Fig. 1. A fragment of rat bladder luminal membrane, obtained by negatively staining fresh scrapings of transitional epithelium with 2 per cent potassium phosphotungstate, pH 7.2. The stain reveals a hexagonal lattice of subunits with a centre to centre spacing of 140 Å–150 Å. ($\times 200,000$.)

Scrapings of fresh, untreated rat bladder membranes were negatively stained with potassium phosphotungstate or ammonium molybdate and examined in either an Elmiskop 1 or AEI 801 electron microscope. Selected negatives were examined in an optical diffractometer. Of the sixty membrane sheets studied (Fig. 1), two were selected for high resolution and sharpness of diffraction spots. Linear integration was carried out in two different directions to obtain the average structure of up to 200 subunits in the lattice. Both the areas integrated showed subunits with twelve regions of high density (Figs. 2-5); the micrograph which showed diffraction spots up to the fourth order (Fig. 2) gave only poor contrast (Fig. 3) while the other which showed diffraction spots up to fifth order (Fig. 4) gave a clearer integrated picture (Fig. 5).

The information obtainable from a micrograph depends on the resolution of the original image. Thus illustration of twelve particles per subunit does not necessarily conflict with earlier reports in which only six were observed. The twelve particles can be seen only faintly if the image



Fig. 2. Optical diffraction pattern of an area of membrane stained as in Fig. 1.

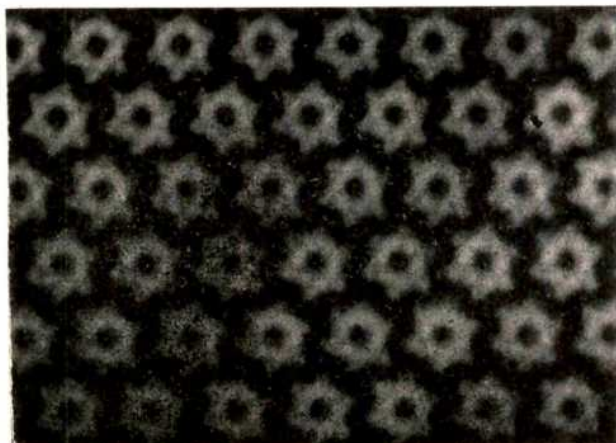


Fig. 3. Area of membrane producing the diffraction pattern of Fig. 2, processed by linear integration in two directions inclined at 60° . ($\times 720,000$.)

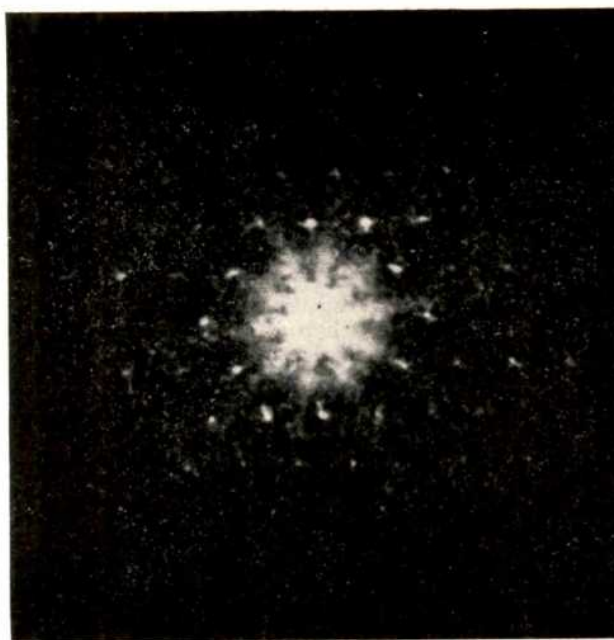


Fig. 4. Optical diffraction pattern of a second area of membrane, stained as in Fig. 1.



Fig. 5. Integrated photograph of the area of membrane producing the diffraction pattern of Fig. 4. ($\times 720,000$.)

gives diffraction spots to less than the fifth order of resolution.

These results indicate that bladder membrane subunits are composed of twelve, rather than six, smaller particles, arranged in a stellate configuration. The subunits are thus dodecamers, not hexamers. As in the mouse bladder membrane³ the subunits are skewed by 19° to the main axis of the lattice.

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Non-solvent Water in Muscle

It is well established¹ that the structure of water in the vicinity of any surface is different from that of bulk water; also, nuclear magnetic resonance and dielectric relaxation studies have shown that, both *in vitro* and *in vivo*, the water adjacent to proteins and other macromolecules is oriented and its motional freedom is impeded²⁻⁶. Furthermore, water exerts a significant stabilizing effect on the structure of some macromolecules⁷⁻⁹. But there is still considerable debate¹⁰⁻¹⁶ concerning (1) the ability of the water of hydration of intracellular proteins to participate in osmotic changes occurring when cells are placed in hyper or hypotonic solution, and (2) the amount of osmotically inactive water in cells in normal physiological conditions. I wish to report a change in the space accessible to dimethyl sulphoxide in isolated guinea-pig intestinal smooth muscle induced by cooling the muscle from +37° C to -7° C in the absence of ice crystal formation. Dimethyl sulphoxide (DMSO) is a polar, water-miscible non-electrolyte which can form hydrogen bonds only by accepting hydrogen (by contrast, water is an acceptor and donor of hydrogen bonds). The kinetics of permeation of DMSO in smooth muscle have been studied previously¹⁷ and this non-electrolyte has been used with some success in preventing damage to smooth muscle exposed to sub-zero temperatures^{18,19}.

Strips of smooth muscle (taenia coli from albino guinea-pigs) were tied at their *in vivo* resting length to quartz frames and their blotted wet weights were measured at various intervals during 30 min of incubation in a continuously oxygenated (O₂/CO₂, 95/5 per cent) Krebs solution at +37° C. One group of muscles was then immersed at +37° C in a Krebs solution containing 20 per cent (w/v) DMSO (BDH, redistilled under vacuum), and a second group was incubated at +37° C in a similar solution containing 5 µCi/ml. of ³⁵S-DMSO (Radiochemical Centre, Amersham). After equilibration for 60 min, muscles were taken from their solutions, surface fluid was removed by a standard blotting technique¹⁷ and the muscles were weighed on a torsion balance. Non-radioactive muscles were then dried to constant weight at 105° C, and the radioactive muscles were placed in 5 ml. of Krebs solution at room temperature and the radioactivity was leached out overnight (there was no increase in the activity of the washout fluid after this period of leaching). Two other groups of muscles were treated in a similar way, but after incubation at +37° C the muscles were cooled to -7° C (that is approximately 0.5° C above the freezing point of the bathing medium) and left for a further hour at this temperature before the usual blotting, weighing and drying (or leaching) procedures were carried out. All solutions were continuously bubbled with the O₂/CO₂ mixture above and below 0° C, and the temperature was controlled to within ±0.5° C. The activity of ³⁵S-DMSO in the washout and loading solutions was assayed by liquid scintillation counting¹⁷ and the density of 20 per cent (w/v) DMSO-Krebs solution was measured at +37° C and at -7° C using a pycnometer with ethanol (Hopkin and Williams analar grade 99.5-100 per cent v/v) as the density standard.

The blotted wet weight of muscles, after 60 min in 20 per cent (w/v) DMSO-Krebs solutions at +37° C was not significantly different from the initial weight of the muscles in isotonic Krebs solution (although there was a transient loss in weight on immersion in the DMSO solution). The space accessible to DMSO represented 78.8 ± 0.7 (n=8) ml./100 g final weight of the muscle (mean ± s.e., n=number of observations), and the dry weight of the muscle together with the DMSO solution within the tissue accounted for its entire wet weight¹⁷. After the extra equilibration period at -7° C, however, the space accessible to DMSO decreased to 71.9 ± 1.2 (n=9) ml./100 g, although the wet weight of the muscles remained unchanged at 99.5 ± 2.2 (n=12) per cent of the initial

value. The density of 20 per cent (w/v) DMSO-Krebs solution increased from 1.0247 ± 0.0003 (n=3) g/ml. at +37° C to 1.0412 ± 0.0007 (n=3) g/ml. at -7° C, and so the space occupied by DMSO at -7° C was equivalent to 74.9 ± 1.2 (n=9) g/100 g, while the solids content remained unchanged at 18.5 ± 1.8 (n=6) g/100 g. Thus there was a significant change ($P < 0.01$ by the *t* test) in the space accessible to DMSO after cooling the muscle from +37° C to -7° C; and, while the water and DMSO spaces were identical at +37° C, the equivalent of 6.6 ± 2.2 g of water for each 100 g of muscle did not act as solvent for the DMSO at -7° C.

Although muscles do not contract in the presence of DMSO at concentrations greater than about 5 per cent (w/v)²⁰, in these experiments muscles suffered no irreversible changes by exposure to DMSO (20 per cent w/v) as judged by their contractile response to histamine when DMSO was removed at +37° C (ref. 19). Furthermore, even though it could be argued that the high concentrations of unlabelled DMSO might have disrupted water structure in the vicinity of proteins—thereby giving apparently equivalent solvent and total water spaces in the muscle at +37° C—preliminary experiments using trace amounts of ³⁵S-DMSO only in the Krebs solution at +37° C (with less than 10⁻⁴ M unlabelled carrier) indicated that the water and DMSO spaces were still identical. This identity between total and solvent water could arise in the unlikely situation that an amount of DMSO equal to that excluded by "bound" water on proteins was adsorbed on to the proteins; in fact, adsorption phenomena usually result in apparently negative amounts of non-solvent water¹¹.

These results support the finding²¹ that the value of Ponder's R (ref. 22) for smooth muscle cells of the guinea-pig taenia coli is unity at +37° C (assuming that collagen in the extracellular spaces provides a negligible contribution to the dry weight of the tissue). Thus at physiological temperatures and tonicities, the concepts of Ling²³ and Troshin²⁴, which involve binding of solutes on to intracellular proteins and low solubility of solutes in intracellular water, need not be used to describe the distribution of ions in smooth muscle²⁵.

The appearance of a non-solvent water fraction at -7° C is not surprising, and a similar tendency has been observed¹³ in human erythrocytes between 25° C and 0° C; however, it is not necessary to assume that this represents a specific conformational change equivalent to low temperature denaturation of proteins²⁶. Any orientation of water, by virtue of its dipole properties, in non-uniform field gradients associated with charged groups on proteins is opposed by the thermal energy of the water molecules, and cooling the system will merely tighten up the structure of the water lattice at the surface of the protein, thereby preventing the probe molecule from being accommodated into the lattice. In this respect measurement²⁷ of the amount of water that cannot be frozen out of solution at -20° C or lower is not a useful criterion¹¹ for non-solvent water in cells at physiological temperatures.

Proteins in muscle constitute almost the entire weight of the dry muscle, and it has been estimated²⁸ that the cell membranes make up only about 0.4 g/100 g wet weight; thus water unable to act as solvent for DMSO at -7° C represents about 0.36 ± 0.12 g/g muscle protein, and it may not be fortuitous that this figure is in line with other estimates of the probable hydration of some proteins²⁹.

Although this and other work^{11,13,14,21} indicates that virtually all cell water is osmotically active in physiological conditions, a temperature-dependent, osmotically inactive fraction of cell water should be allowed for when the osmotic behaviour of cells is considered in the extreme conditions of hypertonicity and temperature encountered during freezing³⁰⁻³². Finally, it should be remembered that any apparently non-solvent water in cells can be

explained in terms either of a lowering of the activity of water in the vicinity of macromolecules or an increase in the osmotic (or activity) coefficient of the macromolecules themselves during the dehydration of cells^{12,33}; on the other hand it could be argued that partial exclusion of solute from cell water provides a physical basis for the thermodynamic concepts.

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Closely Spaced Nodes of Ranvier in the Teleost Brain

ALTHOUGH there have been several studies of internode length of myelinated fibres in peripheral nerve^{1,2} and in main fibre tracts of the central nervous system^{3,4}, there has been only one report of internode lengths within neuropil and nuclear regions of the vertebrate central nervous systems (CNS)⁵. Internode lengths in uninjured peripheral nerves of fishes are always greater than 200 μ m (ref. 1), and studies with rabbit spinal cord suggest that 200 μ m is also a minimum periodicity in main fibre tracts of the CNS⁴. Bodian's study⁵ of internode lengths in CNS included observations of myelinated fibres in the preoptic area, hypothalamus, hypoglossal root and pyramidal tract of the adult opossum. Of fifty-four fibres represented in his chart 1, six had internode lengths of approxi-

mately 100 μ m or less; none had internode distances of less than 50 μ m. During a study of the oculomotor nucleus of the spiny boxfish *Chilomycterus* with the electron microscope, I have made numerous observations of fine myelinated fibres with internodes less than 20 μ m long and occasionally approximately 5 μ m long. Tissue was fixed by perfusion with buffered solutions of osmium tetroxide or glutaraldehyde followed by osmium tetroxide, and prepared for microscopy as reported before⁶.

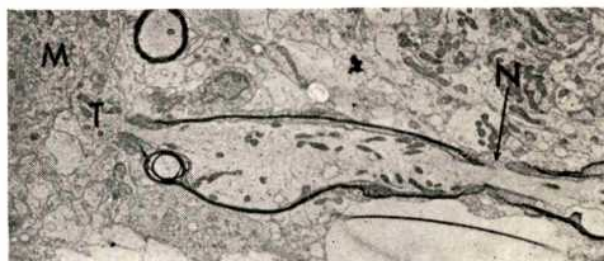


Fig. 1. Electron micrograph from the oculomotor nucleus of the spiny boxfish *Chilomycterus*. A myelinated fibre can be seen crossing the field from right to left. It forms a synaptic terminal (T) in contact with an oculomotor neurone (M). A node of Ranvier (N) is seen, and is approximately 16 μ m distance from the axon terminal. ($\times 3,240$.)



Fig. 2. Electron micrograph of a section of neuropil in *Chilomycterus* oculomotor nucleus. A short myelinated segment, about 6 μ m long, separates two nodes of Ranvier (N_1 , N_2). En passage synapses are established with two adjacent dendritic processes (d_1 , d_2). ($\times 8,400$.)

Nodes of Ranvier were observed frequently in thin sections of the oculomotor nucleus of *Chilomycterus*. Synapses were established at many nodes, as has been reported in other parts of the vertebrate nervous system⁷⁻¹⁰. Synaptic specializations included presynaptic vesicles, thickened membranes separated by a synaptic cleft approximately 20 nm wide, and, at a proportion of synapses, close appositions^{6,11}. The unmyelinated portion of axon at the node could be considerably longer than 1 μ m. Branching of axons was observed at nodes of Ranvier. Some myelinated fibres could be followed for distances of 25 μ m or more along their axes, within a single thin section. Nodes of Ranvier were observed frequently in these longitudinally sectioned fibres, and internodes shorter than 20 μ m were common. Fig. 1 illustrates a preterminal myelinated fibre which forms a synaptic contact with an oculomotor neurone (M). The distance from the node of Ranvier (N) to the axon terminal (T) is 16 μ m. Another example of a short internodal myelin segment is shown in Fig. 2; the distance between nodes of Ranvier is 6 μ m.

On theoretical grounds, Rushton¹² has argued that "it is only possible to argue from one nerve to another if certain proportions exist between their dimensions", and has noted that "nerve fibres do, in fact, exhibit the structural similarity demanded by the theory". Cragg and Thomas¹³ found that the relationship between internode length and axon and fibre diameters, one of the two geometrical conditions required for structural similarity, does not obtain for the lateral line nerve in trout, so that

Rushton's argument does not apply to this nerve. The present observations demonstrate that fine preterminal axons in the teleost brain may deviate considerably, in their pattern of myelination, from peripheral nerve tracts or main fibre tracts of the CNS, so that they do not exhibit "structural similarity" as compared with the latter groups of fibres. It follows that physiological arguments derived from these latter groups of fibres may not be applicable to fibres such as those I have described.

In electron microscopic examination of the oculomotor nuclei and sensorimotor cortex of the cat and rhesus monkey, I have failed to find internodes as short as those I have reported for teleosts. In the absence of precise data on internode distances, however, it is difficult to exclude the possibility of some fibres with internode distances smaller than 200 μm but greater than 20 μm , so that a complete internodal segment within the neuropil would rarely appear in a single thin section. Specification of internode distances, nodal areas and other anatomical parameters of myelinated fibres in neuropil and nuclear regions of the central nervous system will have to be accomplished before it is possible to predict the behaviour of these fibres.

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Projection of the Lateral Eye of *Limulus* to the Brain

THE compound lateral eye of the horseshoe crab, *Limulus*, has been studied intensively for many years¹. It is relatively simple, consisting of a curved layer of interconnected ommatidia—about 1,000 or less in the adult. Each ommatidium contains two types of neurone: a group of reticular cells (ten to thirteen on average) and one, or occasionally two, eccentric cells². Although the axons of both types of cell leave the eye to form the optic nerve, evidence indicates that only the eccentric cell axons discharge nerve impulses in response to light¹. Thus the ommatidia communicate rapidly with the brain only through the impulse activity in the axons of their eccentric cells. (The function of the reticular cell axons in the adult remains obscure. They may have some neurosecretory activity, but apparently do not influence the brain directly by means of electrical signals.)

We describe here the spatial distribution of the eccentric cell axons as they enter the first optic ganglion of the brain. It is a step toward extending the analysis of visual mechanisms in *Limulus* from the eye into the cen-

tral nervous system. Some cells in the brain are known to respond to optic nerve activity with patterns of firing that resemble the activity of vertebrate retinal ganglion cells³; future work will deal with the properties of these and other central neurones.

In Fig. 1, photographs of a lateral eye (A), its optic nerve (B), and a first optic ganglion (C) are accompanied by a drawing that depicts the topographic relationships among these structures. The shape of the first optic ganglion—a thick neural sheet folded on the medial side and open laterally—has apparently not been previously described. With care it can be recognized in properly oriented sections of the brain, but it is often difficult to detect. The form is more easily observed by cutting through the chiasma between the first and the second optic ganglion⁴ and viewing the cut surface of the first ganglion.

Often, as shown here, the optic nerve fibres are grouped into distinct bundles distributed in roughly sequential

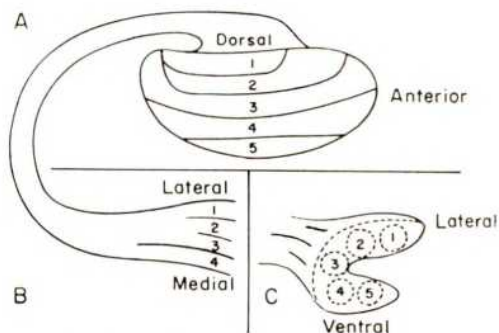
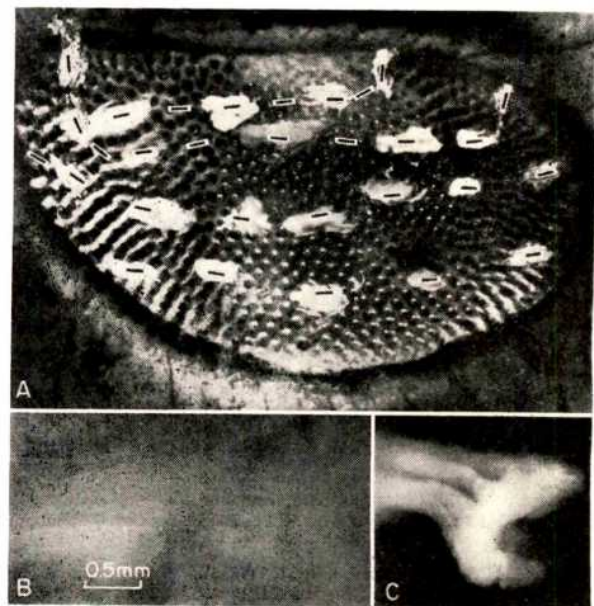


Fig. 1. Mapping of the lateral eye of *Limulus* into the optic nerve and the first optic ganglion. A, Lateral eye with optic nerve (dorsal view) entering the brain in B. This particular nerve is divided into five natural bundles of fibres. Four of the bundles are visible; the fifth lies underneath. C, Proximal view of an isolated first optic ganglion from a different brain. The ganglion is still connected to the optic nerve, but it has been detached from the brain by cutting through the chiasma that joins the first and second optic ganglia. The boundaries of the horizontal bands of ommatidia that contributed active nerve fibres to the five bundles were outlined by placing twenty-three spots of white paint on the cornea; rectangular black-on-white dashes were added to the photograph later for clarity. (See text for details.) The eye is mapped, band by band, into the ganglion in the manner schematically indicated by the line drawing. The curvilinear sequence of circular cross-sections in C is a simplification, for the shapes of the bundles can be irregular, and the positions may be somewhat staggered. The length of the eye in A is about 10.5 mm along the major (antero-posterior) axis. The scale for C is approximately the same as for B.

fashion along the fold of the first optic ganglion. Demoll⁵, who first observed these bundles, saw only three, but we find great variations from animal to animal. In some specimens the optic nerve has no visible divisions, while in others there may be as many as eight or more distinct bundles. What is likely to be important to *Limulus* is not the number of bundles, but the spatial mapping that they establish between the eye and the brain. For the experimenter, however, the bundles provide a "ready-made dissection" of the optic nerve that discloses some aspects of the spatial relationships with particular clarity.

First, one of the lateral eyes, its intact optic nerve, and the forebrain⁶ were excised and placed in a plastic chamber containing Ringer solution (based on measurements of the ionic composition of *Limulus* serum reported in ref. 7; further details in ref. 8). Electrical recordings were made from the optic nerve bundles by means of a suction electrode system. (The device consisted of a disposable syringe (chosen because the rubber plunger provides a good seal) connected through O-ring fittings to a tapered polyethylene tube. A fine silver wire made contact with the Ringer solution that filled the tapered electrode. The use of suction electrodes was described in ref. 9.) Each bundle was cut where it entered the brain and was drawn up into a suction electrode of appropriate size to fit over it. Nerve impulses from the eccentric cell axons in the bundle were amplified and led to a loud-speaker for auditory monitoring. The ommatidia that contributed active fibres to the bundle under study were then stimulated and identified by exploring the eye with a small spot of light.

Three experiments, all giving similar results, were carried out. The first two showed that the active fibres of the optic nerve bundles arose from horizontal bands of ommatidia, and that the bands were arranged in a definite sequence. In the last and most elaborate experiment, the extent of each band of ommatidia was marked on the cornea while observing the eye through a dissecting microscope. The boundary of the band was located by moving a small spot of light exciting no more than two to four ommatidia over the eye until a position was reached where no more responses were evoked in the bundle being studied. This location was marked by a spot of white paint on the cornea, and the procedure was repeated until the outer limits of the band were outlined on the eye. After carrying out this process for each of the bundles (five in this animal) a photograph of the eye was taken (Fig. 1A). We also took photographs showing the optic nerve bundles from this eye (Fig. 1B), and a first optic ganglion from another animal (Fig. 1C).

The relative positions of the optic nerve bundles as they enter the first optic ganglion and the positions of the ommatidia from which they arise are depicted in the line drawings of Fig. 1. All our experiments have shown this same overall pattern. The most dorsal band of ommatidia sends inputs to the most dorso-lateral portion of the first ganglion, the next band of ommatidia projects to the adjacent region, and so on, with the most ventral band of ommatidia sending its active fibres to the most ventro-lateral portion of the ganglion. The net result is that the eye is mapped more or less continuously into the first optic ganglion. A striking feature of this mapping is that incoming fibres from ommatidia separated by a given horizontal distance can be much closer together than fibres from ommatidia separated by the same vertical distance.

So far we have not studied in detail the manner in which the antero-posterior dimension of the eye is represented in the first optic ganglion. But an antero-posterior mapping that occurred within the thickness of the neural sheet, roughly orthogonal to the curve of the fold, would preserve the continuity of the projection.

A second set of experiments was carried out to specify the relationships between the adjacent bands of ommatidia and the adjacent nerve bundles more precisely. With

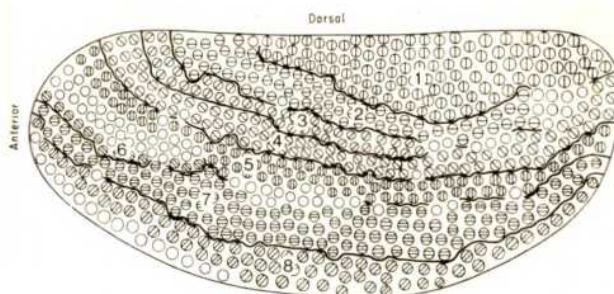


Fig. 2. Drawing of a lateral eye of *Limulus* to show the detailed structure of the horizontal bands of ommatidia that project to natural bundles in the optic nerve. Each ommatidium is represented by a circle in the diagram. In this animal the optic nerve was divided into eight natural bundles. The single or double slash in each circle identifies the particular bundle receiving an active fibre from that ommatidium. An empty circle indicates that no response was recorded from the ommatidium. Definitely established borders between the bands of ommatidia are marked by heavy lines. The ordering of the bands is the same as shown in Fig. 1, with band 1 projecting to the most dorso-lateral portion of the first optic ganglion and band 8 sending fibres to the most ventro-lateral region. The antero-posterior length of this eye was 12.5 mm.

slightly modified techniques (wick electrodes; nervous system exposed but remaining *in situ*) recordings were again made from the natural bundles as they entered the brain. But in this case, the ommatidia were illuminated one at a time by light from a single (76 diameter) fibre optics light guide^{10,11}. The cornea was marked at intervals and notes were made so that each ommatidium could be identified with the nerve bundle containing its eccentric cell axon.

All these experiments gave consistent results; the outcome of one experiment is summarized in Fig. 2. For this illustration the eye was photographed and a tracing was made of the ommatidial array. Each circle represents a single ommatidium, and the slashes within the circles identify the bundle of the optic nerve that received an active fibre from the ommatidium.

There is no overlap between the bands of ommatidia that project to different nerve bundles. Furthermore, some of the bands (for example, four and seven) extend the entire length of the eye without interruption, even though they are as narrow as one or two ommatidia at some points (compare Fig. 1). Within the limits imposed by the irregularity of ommatidial spacing, the boundaries between bands are either nearly straight lines or very gentle curves.

This strict segregation of the visual input into discrete horizontal bands may be associated with the mode of growth of the lateral eye *Limulus*. According to Waterman¹², the lateral eye is roughly circular in young animals, but later grows much more rapidly along the horizontal axis than along the vertical axis. He suggested that this elongation of the eye is accomplished by a proliferation of ommatidia in a growth zone at the posterior margin. Thus the horizontal patterning of the optic nerve projection could be achieved by the simple aggregation of fibres from neurones of a common parentage. If this affinity among growing nerve fibres promoted the establishment of the inhibitory connexions within the eye, then a basis would also be provided for the observed horizontal elongation of the inhibitory fields^{10,11}.

Earlier experiments had already shown that bundles of fibres dissected from the optic nerve near the eye frequently originate in horizontal bands of ommatidia (ref. 11 and unpublished results of B. Knight and F. A. Dodge). This, too, is consistent with the proposal that the horizontal organization is established at the eye, even though it is most easily demonstrated by studying the nerve bundles as they fan out and separate at the brain.

We still do not know whether the horizontal organization of the visual input has some special adaptive value for *Limulus*. It may be related to the immobility of the eyes, which are fixed on the carapace with respect to the ground and the horizon. If so, a similar pattern of pro-

jection might be expected in some of the other arthropods with compound eyes fixed on an immobile head, such as isopods.

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Changes in Behaviour with Repeated Testing under the Influence of Drugs: Drug-experience Interactions

WHEN female rats are injected with a "mixture" of 15 mg/kg of amylobarbitone sodium and 0.75 mg/kg of amphetamine sulphate, and are then placed in a Y-shaped runway, there is a striking increase in their activity, greater than the increases produced by any dose of either drug alone¹. This mixture, although containing a greater proportion by weight of barbiturate, has similar effects to the combination used in the commercial preparation 'Drinamyl'¹. When the procedure is repeated on the same rats over many days there is a decline in the hyperactivity produced by the mixture, which stabilizes after the first few tests at a much lower level².

Several factors could account for this decline. First, the rats may become tolerant to the pharmacological effects of the drug with repeated administration. This seems unlikely, however, for we have not been able to detect any pharmacological tolerance in rats after as many as twenty-five daily injections of this dose of the mixture³. A second possibility is that the decline results merely from repeated exposure of the animals to the same environment, and is therefore a consequence of behavioural habituation⁴. Third, it has been suggested that the decline is due to some form of "behavioural tolerance"⁵. This is usually observed in situations where the effect of a drug is to produce some deficit in behaviour for which the organism can learn to compensate; with repeated testing under the influence of the drug the initial behavioural effect may disappear, although the pharmacological effect can be shown to be unchanged⁶. For behavioural tolerance to occur the organism must experience the drug and the test at the same time; behavioural tolerance can thus be

described as an interaction between exposure to a test and the presence of a drug.

In the experiment reported here we have attempted to assess the contribution of each of these factors to the changes in behaviour resulting from repeated testing with the mixture in the Y maze. To achieve this, the effects of three treatments had to be observed: (1) exposure to the drug without testing (pharmacological tolerance?); (2) exposure to the test without drugs (behavioural habituation?); (3) exposure to both drug and test simultaneously (drug-experience interaction, behavioural tolerance?). The effects of these three treatments had to be compared with the effects of the drug on "naïve" animals which have had experience of neither the drug nor the test. For "behavioural tolerance" to be demonstrated, the decline in activity with repeated testing under the drug should be greater than the decline due to both pharmacological tolerance and behavioural habituation.

Female hooded rats, bred by Animal Suppliers Ltd, were housed five per cage and allowed free access to food (Wyatt's GR/R/3) and water. They were 70–75 days old at the start of the experiment and weighed between 140 and 185 g. There were six groups of ten animals each. Before a final test in the Y maze with the mixture, four of the groups were pretreated as follows: groups 1 and 2 received twenty-four daily injections of either the mixture (group 1) or saline (group 2) after which they were returned to their home cages. Groups 3 and 4 were given twenty-four daily trials in the Y maze preceded by an injection of the mixture (group 3) or saline (group 4). Group 5 was given twenty-four daily saline injections and group 6 had twenty-four daily trials in the maze, preceded by saline injections. Both groups were therefore handled; they were given saline on the test day and thus served as controls. All injections were given subcutaneously 35 min before a trial in the maze. The mixture was given in the same dose as used previously^{2,3}, 15 mg/kg of amylobarbitone sodium and 0.75 mg/kg of amphetamine sulphate in the same solution. We allowed 1 day without any trials or injections between the end of the pre-treatment phase and the final test in the runway in order to minimize the possibility that any drugs previously injected were still present⁷. The testing procedure has been described before^{1,3}. A trial lasted 3 min during which the number of entries into an arm of the maze, rears, faecal boluses and urinations was counted. Our discussion is confined to entries which yielded the most consistent and reliable results¹. An entry was scored if all four feet of the rat entered an arm.

Fig. 1 shows that there was a decline in the activity of group 3, drugged and tested in the Y maze for twenty-five trials, but it was not very marked ($F=2.55$, d.f.=4,36, $P<0.10$). Indeed, on the test day these rats were slightly but not significantly more active ($t=1.45$, d.f.=18) than rats which had neither been in the maze nor had any previous injections of the mixture (group 2), whereas their activity would be expected to be below that of group 4 on the test day if behavioural tolerance had developed. Comparison of group 2 and group 1, pretreated with the mixture for 24 days, shows that the latter were no less active under the influence of the mixture on the test day than animals receiving the drug for the first time ($t=1.19$, d.f.=18); that is, no pharmacological tolerance was evident, which confirms our previous finding³.

Exposure to the Y maze without drugs, however, drastically reduced the drug response (groups 2 and 4: $t=3.78$, d.f.=18, $P<0.001$). Group 4 showed only a slight increase in activity when given the mixture on the test day (groups 4 and 6: $t=1.85$, d.f.=18, $P<0.05$). Their score was not different ($t=0.33$, d.f.=18) from that of undrugged animals introduced to the maze for the first time (group 5). Rats of group 5 were significantly more active than those of group 6, with previous experience of the Y maze without drugs ($t=1.97$, d.f.=18, $P<0.05$). This result is evidence that behavioural habituation⁴

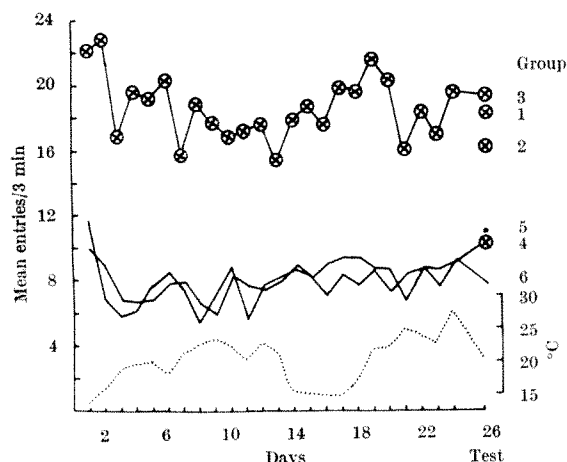


Fig. 1. Y maze activity of different groups of rats. Each point is the mean of results for ten rats. ○, The mixture (15 mg/kg of amylorbarbitone sodium and 0.75 mg/kg of amphetamine sulphate). ●, Saline. Groups 3, 4 and 6 were injected as indicated and given twenty-five daily trials in the Y maze. The remaining groups received twenty-four daily pre-test injections of either the mixture (group 1) or saline (groups 2 and 5) and were tested only on day 26 after receiving the drugs indicated. There was 1 day without injections or trials between the end of the pre-test phase and the test day. ····, Daily temperatures during the course of the experiment (June 23–July 18, 1969).

had occurred in group 6. These findings are very similar to those obtained in studies of the effects of a single Y maze experience, with or without the mixture on reactions to the drug on a subsequent trial⁸.

In our results rats of group 2 were considerably less active on the test day after receiving the mixture than group 3 on the first day of the experiment, although both groups were receiving the mixture and performing the test for the first time; there was no difference between the corresponding saline groups (group 5 compared with groups 4 and 6). The interaction drug condition \times days is significant ($F = 11.59$, d.f. = 1, $P < 0.01$) and may be partially due to differences in temperature on respective days (13.67°C on day 1; 20.33°C on the test day, Fig. 1). Rats appeared to be less active and exhibit more ataxia on hot days (above 20°C). Thus differences in temperature may perhaps account for some of the changes in activity during the experiment.

In summary, we have found no evidence of pharmacological or behavioural tolerance. There was certainly an interaction between exposure to the Y maze and the presence of the drug (groups 1, 2, 3, 4: $F = 6.61$, d.f. = 1, 36, $P < 0.02$), but it was in a direction opposite to that required for behavioural tolerance; repeated testing with the mixture in the Y maze did not lead to any great reduction in activity, whereas exposure to the Y maze without the drug resulted in almost complete abolition of the drug response.

The problem posed is why this should be so. A possible explanation is that the mixture interferes with behavioural habituation⁴. Consistent with this interpretation is the finding that the decline in activity within a single Y maze trial is much less steep with the mixture than with saline¹. If behavioural habituation is retarded by the mixture, sustained activity in the presence of the mixture and a reduced effect of the mixture after behavioural habituation has developed would both be expected.

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Rapid Eye Movements and Remembering

SOME theorists have ignored the content of dreams and concentrated specifically on chemical or physiological aspects¹. Oswald² has postulated that rapid eye movement (REM)³ sleep is a non-specific indication of many forms of synthesis within cerebral neurones, and that periods of massive learning would cause high percentages of REM sleep. Others have explained both dream content and REM state as playing a part in information processing. Some have used computer analogies, suggesting that reorganization takes place during the REM state, involving revision and updating of cognitive processes and memories⁴. It is known that remembering involves distortion and conventionalization⁵, so REM-induced reorganization should also show "effort after meaning".

Sleep has been shown to favour retention⁶ but it is not known how. If REM sleep is a positive process of consolidation and does not merely protect the trace from new input (as the "interference theory" of forgetting would predict) it might provide either a simple "setting" of the trace or an active reorganization and updating. If we measure the amount recalled by REM-deprived (RD) and non-REM-deprived (NRD) subjects and find that the latter retain more after sleep, it would support positive action theories against interference theories. If we examine the amount of organizational distortion and find more in the NRD subjects it would offer support to reprogramming as opposed to "setting" theories.

Our experiment involved ten pairs of male student volunteers, spending their second night in adjacent laboratory beds. At 2100 h the subjects listened to a tape recording of the material to be remembered. After lights-out one subject was randomly assigned to be REM-deprived, and whenever he showed signs of the REM state (according to a standard manual⁷) both were woken up, and kept awake for 5 min. The subjects got up at 0700 h and were immediately asked to recall in writing all the material learned the night before. This consisted of a list of thirty-two nouns, five sentences and a prose passage. The list was compiled from four categories (animals, vegetables, parts of the body and birds) arranged to give few consecutive category repetitions⁸. The five sentences were syntactically correct but meaningless (for example, "The academic liquid attended a deep bar")⁹. The list and the sentences were presented five times each, followed by two readings of a 162-word prose passage, specially written to contain several anomalies.

As intended, the principal effect of the regime was a very marked decrease in REM sleep in the RD subjects. They had, however, slightly less sleep altogether, and slightly more slow wave sleep (Table 1).

The amount of recall achieved by the two groups in the morning showed systematic differences: the RD group recalled less of all three sorts of material than the NRD group (Table 2). This effect was most marked in the recall of the prose passage, in both number of words used and accuracy scores—means of scores given by five "blind" independent judges, who agreed very highly significantly

Table 1. MEANS AND STANDARD DEVIATIONS OF THE TOTAL TIME (IN MINUTES) OF SLEEP STAGES ACHIEVED BY SUBJECTS IN THE TWO GROUPS

		Onset of sleep	Wake	I	II	III	IV	FEM Sleep	III +IV
RD group	\bar{x}	34.1	151.4	73.5	159.7	22.3	75.1	7.2	337.8
	s.d.	13.59	50.64	44.01	38.41	11.40	20.22	5.60	49.21
NRD group	\bar{x}	30.4	125.3	72.7	174.6	19.5	55.6	45.7	368.1
	s.d.	15.02	37.38	44.38	26.26	10.22	21.28	19.36	37.42
t (matched pairs)		0.61	1.93	0.05	1.25	0.49	2.46*	5.46†	2.18

* $P < 0.05$.† $P < 0.001$ (two-tailed).

Table 2. RECALL SCORES

Word list	No. correct	Clustering score	
RD group \bar{x}	16.6	3.16	
s.d.	4.88	3.97	
NRD group \bar{x}	18.3	4.45	
s.d.	5.29	6.42	
t (matched pairs)	1.14	0.44	
Sentences	No. of words correct	Restructuring score	
RD group \bar{x}	11.8	0.31	
s.d.	7.63	0.29	
NRD group \bar{x}	17.6	0.18	
s.d.	3.41	0.04	
Wilcoxon T	6*	17.5	
Prose	No. of words	Accuracy score	Distortion ratio
RD group \bar{x}	91.7	79.8	0.51
s.d.	28.28	30.54	0.31
NRD group \bar{x}	119.7	116.8	0.29
s.d.	23.11	33.49	0.15
(matched pairs)	2.85†	2.82†	2.77*

* $P < 0.05$.† $P < 0.02$ (two-tailed).

with each other when asked to rate the material in a systematic way¹⁰.

Measures of restructuring of the word list¹¹ and sentences were both in the "reprogramming" direction, but not statistically significant. The RD group who remembered little of the prose passage, however, showed relatively more distortion than the controls.

Unfortunately, the difference in amount of sleep between the groups could have accounted for the differences in recall on an interference theory. The result of a conservative analysis of covariance, however, shows that the accuracy measure for the prose remains significant at the $P < 0.05$ level (two-tailed) when the variance attributable to amount of sleep has been taken into account.

It seems very likely, in the light of these results, that the REM state has an important part to play in the consolidation of memories, and that possible differences in restructuring remain worth pursuing.

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Simultaneous Acoustic and Semantic Coding in Short-term Memory

It has been suggested^{1,2} that memory for verbal material comprises two components, one of which is labile and depends on the acoustic properties of the words while the other, which is more durable, is based on their meaning. The observed relationship between rate of forgetting and type of coding is explicable in at least the three following ways. (a) Material may pass from a short term store which uses an acoustic code into a long-term semantically coded store. (b) Material may be encoded on input either acoustically, in which case rapid forgetting occurs, or else semantically, in which case forgetting is relatively slow. (c) Material may be encoded both acoustically and semantically on input, in which case immediate recall will show the effects of both methods of encoding, but because the effects of acoustic coding are short lived, delayed recall will show only semantic effects.

The following experiment aims to decide between the three hypotheses. Subjects tried to remember sequences of three words which were either similar or dissimilar in sound and which either made up a meaningful phrase or were unrelated. The combination of two levels of acoustic similarity with two levels of semantic compatibility thus gave four basic conditions, similar and compatible, for example, "I might fly"; similar and incompatible, for example, "eye fight dry"; dissimilar and compatible, for example, "we could stop"; dissimilar and incompatible, for example, "king wake slit". Subjects attempted recall after either 2 or 20 s during which they were required to perform a distracting task. We assumed that a considerable short term component would be present after 2 s but would have dissipated after 20 s of distraction³. Subjects were successively presented with two sequences of three words on each test trial, the sequences being drawn from the same condition, and in the similar conditions they were acoustically similar to each other; for example, "Jude chewed food, lewd dude wooed". Each word was presented visually for 1 s.

The acoustic similarity effect is typically reflected in the difficulty of recalling the order in which items were presented rather than in recall of the items themselves, which may indeed be enhanced by acoustic similarity⁴. Item recall was not therefore required, the six relevant words on each trial being written in random order on a prompt card which was displayed during recall. To ensure that subjects could not respond correctly merely by rearranging the compatible sets to form meaningful triads, sets of three words were selected, which were comparatively meaningful in any of the six possible permutations of order, for example, "I might fly"; "might I fly"; "fly I might" and so on. Six subgroups of subjects were tested so that the six permutations of each triad were used once. For the intervening task, the experimenter read out random digits at a rate of one per second and subjects were required to add one to the digit and write down the answer. After two or twenty such digits they were given a verbal recall signal and shown the relevant prompt card, whereupon they attempted to write down the two word triads in the correct order. After 20 s the signal "ready" was given and the next trial began. The order of presentation of the four conditions and two delays was random. Six groups of five subjects were tested.

Performance was scored in terms of the number of words recalled in the appropriate serial position. The results (Table 1) were analysed using the Wilcoxon test with two-tailed significance levels. There was a reliable effect of semantic compatibility at both the 2 s ($t = 13$, $P < 0.01$) and the 20 s delay ($t = 8$, $P < 0.01$). The effect of acoustic similarity, however, occurs only at the short retention interval where it is shown by both compatible ($t = 45$, $N = 24$, $P < 0.01$) and incompatible triads ($t = 88$, $N = 27$, $P < 0.02$). At the longer delay, neither compatible nor incompatible triads show an acoustic similarity effect.

Table 1. MEAN PERCENTAGE OF WORDS RECALLED AS A FUNCTION OF ACOUSTIC SIMILARITY, SEMANTIC COMPATIBILITY AND RETENTION INTERVAL

Condition		Delay		Percent forgotten ($\frac{2\text{ s}-20\text{ s}}{2\text{ s}} \times 100$)
		2 s	20 s	
Compatible	Similar	61.0	68.9	-13.0
	Dissimilar	71.8	68.2	5.0
	Difference	10.8	-0.7	
Incompatible	Similar	42.8	40.9	4.4
	Dissimilar	52.2	46.0	11.7
	Difference	9.4	5.1	

Only the incompatible dissimilar triads show significant forgetting during the 20 s delay ($t = 90$, $N = 27$, $P < 0.05$), while triads which are both compatible and acoustically similar show a significant improvement in performance after the 20 s delay ($t = 60$, $N = 25$, $P < 0.01$).

These results are consistent with the hypothesis of simultaneous semantic and acoustic coding. Recall after 2 s uses both sources of information even though this is a sub-optimal strategy for one of the four conditions (acoustically similar compatible). Because acoustically coded information is rapidly forgotten, recall after 20 s depends chiefly on semantically coded information and the adverse effects of acoustic similarity are therefore absent.

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Axonal Transport of Neurotubule Protein

It has been known for some time that there is a constant somatofugal transport of materials down axons¹, but it has only recently been clearly demonstrated that more than one rate of transport may exist, and that transport cannot therefore be considered to be a single process²⁻⁴. As far as transport of protein is concerned, at least two mechanisms seem to be operating, at significantly different rates. First, there is a "fast" protein component, which, as our previous studies have shown, advances at a rate of about 50 mm per day in goldfish optic nerve⁵; its counterpart in mammalian nerve has been found to have a rate of up to about 500 mm per day^{6,7}. Second, there is a "slow" component whose rate of advance is about two orders of magnitude slower; the value in goldfish optic nerve was found to be about 0.4 mm/day (ref. 8), while in mammalian nerve, values of 1-10 mm per day have been observed². It is clear, moreover, that the fast and slow types of transport involve different cellular constituents, for the fast component consists almost entirely of insoluble—that is, particulate—protein, while the slow component contains about 40-50 per cent soluble protein^{5,9}.

We have now attempted to establish to what extent the neurotubule protein might be transported with either of the two components of protein transport. We took advantage of the fact that colchicine binds to neurotubule protein as it does to other types of microtubules, such as those in mitotic spindles, sperm tails and cilia¹⁰. We injected 2 μ Ci of [methoxy-³H]-colchicine (specific activity 5 Ci/mmmole) with a microlitre syringe into the posterior chamber of one eye of a series of goldfish of 5-7 cm in length. Both optic nerves and both optic tecta were then dissected out at appropriate intervals, the samples were dried and weighed, and tissue radioactivity was deter-

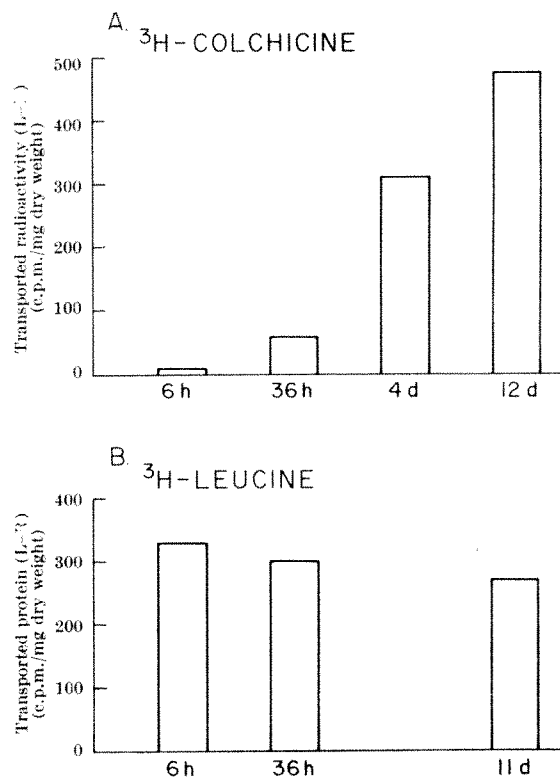


Fig. 1. A. Transported radioactivity in optic tectum after injection of 2 μ Ci ³H-colchicine into the right eye. B. Transported protein in tectum after similar injection of 4 μ Ci ³H-leucine. Each value is the difference between the left and right tecta in c.p.m. per mg dry weight, and represents the average obtained from five animals.

mined by an incineration technique¹¹. As has been shown⁵, a considerable proportion of the injected material is rapidly absorbed into the bloodstream, and gives rise to a certain level of "background" radioactivity on both sides of the brain. But only the optic nerve and tectum contralateral to the injected eye contain the labelled transported material, which is measured as the difference in "specific activity" (c.p.m. per mg dry weight) between the two nerves or two tecta. To determine the rate of protein transport, we injected 4 μ Ci of 4,5-³H-leucine (specific activity 5 Ci/mmmole) as above, and treated the tissue samples with either 10 per cent trichloroacetic acid or with Bouin's fixative to precipitate the proteins before incineration.

In Fig. 1, the time course of arrival of the transported labelled material after ³H-colchicine had been injected (1A) is compared with the time course of arrival of labelled protein after injection of ³H-leucine (1B). It is clear that the early appearance of labelled protein, which corresponds to the arrival of the fast component of protein transport, does not have any counterpart in the case of colchicine. At 24 h after the injection, for example, there is almost no transported colchicine.

Because the neurotubules may become disrupted when colchicine binds to them¹² it might be possible to ascribe the observed difference in the rates of arrival of labelled protein and colchicine to the fact that protein transport had been reduced in the presence of colchicine. Indeed, it has been reported that high levels of colchicine injected into a nerve may block fast protein transport entirely¹³⁻¹⁵. We found, however, that even at colchicine levels of 1 μ g per eye—about three times as high as those used in the experiment with labelled colchicine—neither fast nor slow protein transport was markedly affected (Table 1).

To determine whether the transport of labelled colchicine might be the result of a non-specific process such as diffusion or "interfacial creep"¹⁶, we tested for retrograde

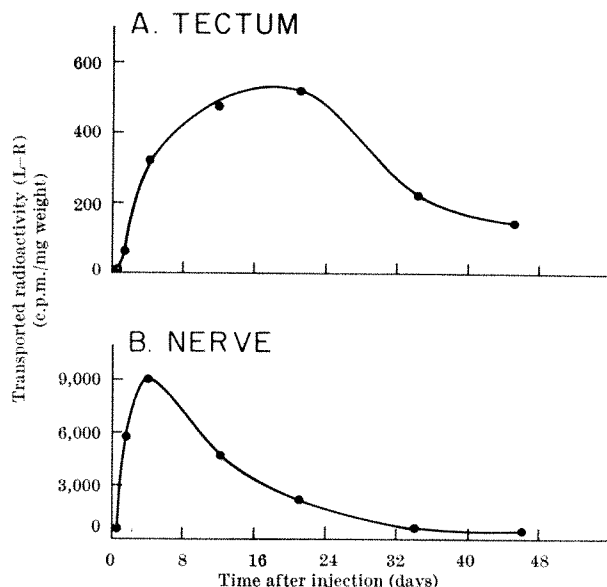


Fig. 2. Transported radioactivity in (A) optic tectum and (B) optic nerve after intraocular injection of ^3H -colchicine. Each point represents the average obtained from five animals.

transport, that is from tectum to retina. We injected 2 μCi of ^3H -colchicine intracranially, after crushing one optic nerve, so that although the injected material had access to the terminations of the optic nerves in both tecta, the transport of labelled material from tectum to retina could occur only on the side on which the nerve was intact, and would be expected to reveal itself as a difference in activity between the two retinas. Four days after the injection no such difference between the retinas was seen and it is unlikely, therefore, that a non-specific physico-chemical process could account for the transport of colchicine. Thus we presume that the colchicine was transported in association with the neurotubule protein.

To determine the rate of this transport, we compared the time courses of appearance of labelled colchicine in the nerve and in the tectum (Fig. 2). In the nerve, a peak in radioactivity was seen at about 4 days (2B), whereas in the tectum a much broader peak was seen at about 2-3 weeks (2A). The distance traversed from the middle of the nerve segment to the middle of the tectum was about 7 mm, so it can be estimated that the peak of the mass of radioactive material was advancing at a rate of 0.4-0.7 mm per day, which is clearly consistent with the previous estimate⁸ of 0.4 mm per day for the peak of the slow component of protein transport.

To confirm that the neurotubule protein was part of the slow component, we tried to isolate this protein from nerves which had previously been labelled by intraocular injection of leucine, at a time when the labelling of the slow component in the nerves was known to be high—at 6-16 days after the injection. Two different techniques were used, including ammonium sulphate precipitation followed by extraction on DEAE-Sephadex¹⁴, and precipitation with vinblastine¹⁸. Both gave disappointingly low yields with goldfish nerve, but when rat nerve

was used it was found that about 15 per cent of the soluble labelled protein in the nerve could be accounted for as neurotubule protein. (This is about as much labelled soluble neurotubule protein as can be extracted from the whole adult mouse brain 24 h after injection of labelled amino-acid¹⁹.) It is likely that the insoluble protein, which constitutes at least 50 per cent of the slow component^{5,9}, also contains an appreciable proportion of neurotubular material, and in the goldfish most of the neurotubule protein may be in the insoluble compartment.

Thus neurotubule protein is transported at the rate of the slow component of protein transport, but it does not constitute the whole of that component. This observation is particularly interesting in view of the suggestion²⁰ that the neurotubules might play a part in mediating fast transport. They evidently do not themselves move at the rate of the fast transport.

We have previously summarized evidence for the view that the material involved in the fast transport consists of vesicles and/or mitochondria⁵. It does not seem likely therefore that the formation of such vesicles could occur by the pinching-off of portions of the neurotubules, as some²¹ have suggested.

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Table 1. EFFECT OF COLCHICINE ON ACCUMULATION OF RADIOACTIVE PROTEIN AFTER INTRAOCULAR ^3H -LEUCINE INJECTION

Colchicine injected (μg)	"Fast transport" (tectum 6 h after injection) (c.p.m./mg) (per cent)		"Slow transport" (optic nerve 12 days after injection) (c.p.m./mg) (per cent)	
—	208	100	1,597	100
10^{-4}	176	85	2,020	126
10^{-3}	172	83	1,646	103
1	207	100	1,307	82

One μl . of the colchicine solution in isotonic saline was injected into each eye 2 h after 4 μCi of ^3H -leucine was injected into the right eye, so that leucine incorporation was not interfered with. All values represent the difference between the labelled and unlabelled sides, in c.p.m./mg dry weight, and were obtained by pooling data from 3-5 animals in each case.

Psychological and Sociological Investigation of *XY* Prisoners

CYTOGENETIC surveys (Table 1) suggest that males with the sex chromosome constitution *XY* tend to be concentrated among tall detained delinquents, especially those with psychiatric disorder. We have carried out a survey of tall prisoners at a London prison, with a population which, although heterogeneous, is largely composed of recidivists from London and the Home Counties. For practical purposes we had to divide the surveyed popula-

tion, all of whom agreed to take part in the project, into the following groups. (1) Prisoners 6 feet tall and over, admitted to the prison in 1967 during limited periods governed by the availability of laboratory and other services. (2) Randomly selected prisoners 5 feet 11 inches tall and over, who were resident in March 1968. (3) Prisoners 5 feet 11 inches tall and over, admitted between September 15, 1968, and October 18, 1969.

Table 1. PREVIOUS CYTOGENETIC SURVEYS

		No.	XYF	Per cent
Prisoners*	Unselected ^{1,2,†}	939	1	0.1
	Tall ^{2-4,†}	431	16	3.7
Criminals with psychiatric disorder (usually cared for in special institutions)	Unselected ^{5,10-14}	931	17	1.8
	Tall ^{2,5,6,13,15}	612	38	6.2
Psychiatric patients	Unselected ^{8,14,16}	2,070	3	0.1
	Tall ^{8,13,17,18}	247	7	2.8
Newborn males ^{19-21,‡}		8,725	12	0.13

Ref. 21 refers to random selection of healthy infants.

* These prisoners include detained juvenile delinquents.

† Unpublished results of P. A. Jacobs *et al.*

‡ Personal communication from H. A. Lubs and F. H. Ruddle.

Leucocytes were cultured from 10 ml. of venous blood by Moorhead's²² method, air-dried and stained with Giemsa. Results are given in Table 2.

Table 2. INCIDENCE OF KARYOTYPE 47 XYF

Group	Specimens successfully cultured	Karyotype 47 XYF
1	34	2
2	31	1
3	290	6

Each subject with an XYF constitution was matched, from the same group, with a control of normal karyotype whose age was within 12 months and height within 3 inches of the subject, and who had been admitted as near as possible to the time when the subject had entered the prison. Ages ranged from 23 to 46 yr with a mean of 30. The mean height of subjects was 73.4 inches and of controls was 72.3 inches; six out of nine subjects were taller than their controls.

Sociological and criminological information about the prisoners was obtained from a standard questionnaire supplemented by police, prison, parole and probation records. As far as possible, data were expressed quantitatively and certain variables were used to construct rating scales. Subjects and controls were also examined physically and as many as possible were tested on the Wechsler adult intelligence scale; the Eysenck personality inventory (PEN); and the Foulds and Caine extrapunitive intrapunitive scale.

Table 3. INTELLIGENCE QUOTIENTS

Pair No.	Verbal		Performance		Full scale	
	XYF	Control	XYF	Control	XYF	Control
1	91	101	91	102	90	101
2	97	116	90	108	93	114
3	94	102	93	97	93	99
4	91	96	85	98	87	97
5	88	107	97	108	91	108
6	102	127	98	124	100	127
7	103	100	89	120	97	109

Table 4. EXTENT OF PREVIOUS PSYCHIATRIC HISTORY

Pair No.	XYF	Control
1	3	1
2	1	0
3	2	0
4	1	1
5	3	2
6	2	0
7	2	1
8	3	1
9	1	1

Four subjects but no controls had received treatment in mental hospitals.

Differences between pairs were evaluated by the Wilcoxon two-tailed signed ranks test. Differences at the 0.02 level of significance were found in respect of intelligence levels (Table 3) and the extent of previous psychiatric history (Table 4). The latter was estimated in numerical form by scoring each prisoner as follows:

for a history of psychiatric illness requiring institutional treatment, 3; for a history of psychiatric illness requiring only out-patient treatment, 2; for a history of untreated psychiatric disturbance, 1; and for no history of psychiatric disturbance, 0.

Differences at the 0.05 level of significance were found in respect of the number of previous convictions (Table 5) and a relative achievement scale (Table 6), the latter being a simply calibrated numerical expression of each prisoner's scholastic achievement and employment level relative to his father and brothers. Differences approaching this level of significance were found in respect of the number of previous accidents, the sum of theft and break-in offences and a sociability scale (Table 6) of eight questions bearing a substantial relationship to sociability, each item being scored as 0 in the direction of solitariness or 1 in the direction of sociability. This difference in sociability may be reflected in the extraversion scores of the PEN questionnaire (Table 7). Certain interesting data, although unproductive of significant differences, are given in Table 5.

Table 5. MISCELLANEOUS DATA ABOUT PRISONERS

	Mean values	
	XYF	Controls
No. of siblings	2.9	3.3
Birth order	2.5	2.6
Paternal age at prisoners' birth (yr)	33	30.5
Maternal age at prisoners' birth (yr)	28	25.8
No. of jobs	16.1	11.4
Longest job (months)	22.1	22.8
Maximum weekly earnings (pounds)	33.8	37.6
No. of serious accidents	1.3	0.4
No. of children	1.4	0.7
Age at first conviction (yr)	16.7	16.1
No. of offences	31	34.5
No. of times convicted	13	9.2
Total length of previous sentences (months)	56	62.3
No. of offences by category:		
Drink or drugs	0.3	0.2
Fraud	0.3	14.8
Theft	13.7	10.6
Taking and driving away	2.8	2.3
Breaking-in	4.8	3.0
Violence (property)	0.4	0.2
Violence (person)	0.3	0.8
Sexual offences	0.3	0

Six out of seven subjects were taller than their fathers compared with four out of seven controls. Five out of five subjects were taller than their brothers, compared with five out of eight controls.

Table 6. SOCIABILITY SCORE AND ACHIEVEMENT RELATIVE TO FATHER AND BROTHERS

Pair	Achievement		Sociability	
	XYF	Control	XYF	Control
1	-1	+1	3	7
2	-2	+1	0	6
3	-1	0	4	6
4			7	7
5	-3	-2	2	5
6	-1	-1	5	7
7	-2	0	5	5
8	-1	-1	0	4
9	-1	0	5	3
Total	-12	-2	31	50

Table 7. EYSENCK PERSONALITY INVENTORY

Pair	Psychoticism	Extraversion	Neuroticism
1	8 (9)	7 (13)	8 (6)
2	16 (5)	7 (14)	17 (4)
3	13 (13)	15 (17)	21 (7)
4	5 (16)	13 (14)	8 (16)
5	9 (3)	8 (16)	12 (15)
Total	51	50	66
Mean	10.2	10	13.2

Control values given in parentheses.

Differences between family and school history of subjects and controls were appreciable but not statistically significant. One subject and three controls had a family history of psychiatric disorder; one or both parents of seven subjects and two controls had a history of alcoholism, and three subjects and four controls had a near relative who had been convicted of a criminal offence. Four subjects had attended schools for maladjusted or difficult children; none of the controls had been to such a school. Four subjects and one control had a record of truancy, and three subjects and one control had been expelled from school.

The comparison of employment between subjects and controls was mitigated by the large number and variety of jobs and a considerable degree of discrepancy between the various records. There remained a strong impression, however, that in general subjects were doing considerably less skilled work than the controls. The magnitude of the recorded discrepancies revealed no significant differences between subjects and controls.

Two subjects and one control could be regarded as heavy drinkers; three subjects and two controls had taken drugs but none could be described as addicts. There were no serious gamblers in either group. Six subjects and three controls had no established sexual partner. No subject or control professed homosexual tendencies but they were mentioned in the records of three subjects. Four subjects but no controls admitted having few or no girl friends before marriage.

Physical examination revealed tremor in two subjects, nystagmoid movements in three and stammer in two. Such features were not apparent in the controls apart from one case of tremor. Two subjects had pes cavus, and one slight facial hemiatrophy.

The conclusions are mitigated by the small number of subjects examined, the unassessed reliability of sociological data and the heterogeneity of controls. Nevertheless, we gained the impression that the XYY constitution in prisoners may be accompanied by the following tendencies (the first five of which occur with statistically significant frequency): (1) a past history of mental illness, often severe, and usually designated psychopathy; (2) lower than average intelligence; (3) higher frequency of convictions; (4) less achievement in comparison with father and brothers; (5) greater height in comparison with father and brothers; (6) a criminal history of offences against property; (7) asocial personality; (8) severe alcoholism of ascendants, usually father; (9) homosexuality, covert rather than overt. The degree of interdependence of these tendencies must remain speculative until a larger sample is available for analysis. The XYY constitution, however, has acquired some measure of definition and it might be profitable to explore its biochemical aspects in greater detail.

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Abrin and Ricin: New Anti-tumour Substances

Abrin and ricin are highly toxic proteins isolated and crystallized from *Abrus precatorius*¹ and *Ricinus communis* respectively²⁻⁴. The LD₅₀s of abrin and ricin are 0.020-0.012 mg/kg body weight of mice respectively. Both have a molecular weight of 65,000, but a different chemical structure as judged by the analysis of N and C terminal amino-acid residues. For abrin these are leucine⁵, while the N-termini of ricin are alanine and isoleucine, and the C-termini are phenylalanine and serine^{2,6}. The biological activities of the two proteins are alike; both cause similar toxic symptoms and have no effect on mitochondrial respiration^{7,8}. The inhibitory effect of abrin and ricin on Ehrlich ascites tumour has been demonstrated by simultaneous injection of a sublethal dose with the tumour cells. Total inhibition of tumour growth in mice was demonstrated by measurements of body weight and total cell numbers in the peritoneal cavity. The therapeutic index of abrin was found to be 4. These inhibitory effects can be eliminated by heating the phytotoxin solution at 100°C for 30 min^{9,10}, which indicates that the anti-tumour activity of phytotoxins is due to protein.

We have used mice bearing Ehrlich ascites tumours, of various days' duration, to study the anti-tumour activity

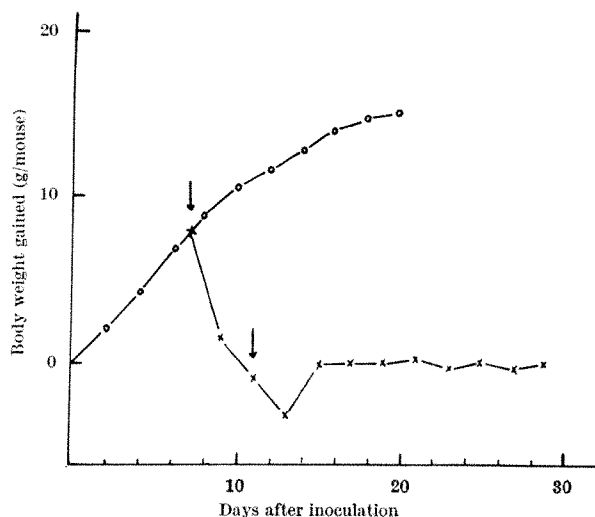


Fig. 1. Effect of abrin on the change of body weight of mice given 2×10^7 Ehrlich ascites tumour cells and two single doses of abrin (0.2 μ g/mouse), each on days 8 and 11 as arrowed.

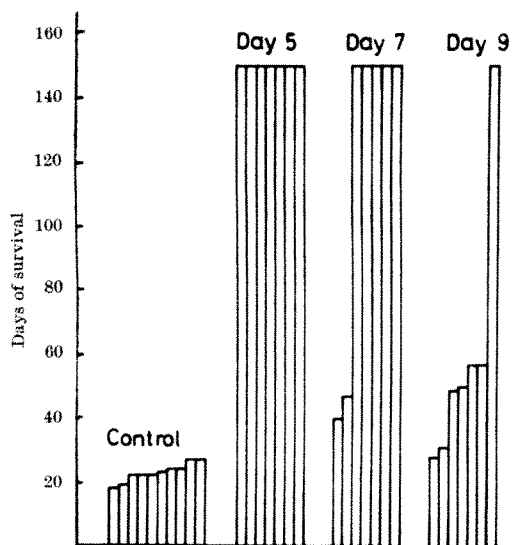


Fig. 2. Survival of mice treated with saline and abrin (injected on days 5, 7 and 9). A second injection was given on the third day after the first treatment. Each bar represents one animal.

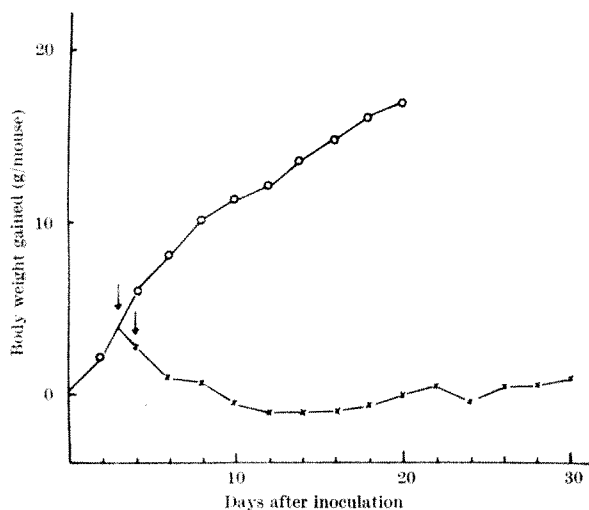


Fig. 3. Effect of ricin on the changes in body weight of mice given 2×10^7 Ehrlich ascites tumour cells and two single doses of ricin ($0.2 \mu\text{g}/\text{mouse}$), each on days 3 and 4 as arrowed.

of abrin and ricin. The two toxins were dissolved in isotonic saline for injection. Although, when tumours develop, the weight of the mice increases by several grams, we used 20 g as the basis for our calculation of the dose in $\mu\text{g}/\text{kg}$ body weight.

In all experiments, NMRI male mice, average weight 20 g, were inoculated intraperitoneally on day 0 with 2×10^7 Ehrlich ascites tumour cells with 98 per cent viability demonstrated by the eosin Y exclusion method¹¹. A solution of abrin or ricin was injected intraperitoneally into the mice on days 1, 3, 5, 7, 8, 9 and 11. As a control, isotonic saline was injected after inoculation with tumour cells. Results were evaluated on the basis of a comparison of the average change in body weight of experimental and control mice.

When treatment of tumour bearing mice with a single sublethal dose ($10 \mu\text{g}/\text{kg}$ body weight) was initiated on days 1 and 3, the growth of Ehrlich ascites tumour was completely suppressed. Mice treated with abrin lost weight on the first day after treatment and then gradually gained weight to the level of mice not inoculated with tumour cells. Two doses of $10 \mu\text{g}/\text{kg}$ body weight were required to suppress the growth of tumour completely if the treatment was initiated on days 5 and 7. The second

dose was injected 3 days after the first. The results are summarized in Figs. 1 and 2. Once the tumour was cured, the mice survived during 50 days of observation and no tumour cells were detected. When toxin was injected on days 9 and 11 no complete cure of tumour was observed.

Ricin had a similar inhibitory effect on Ehrlich ascites tumour. For complete inhibition of tumour growth, a single sublethal dose of $7.5 \mu\text{g}/\text{kg}$ body weight was required on days 1 and 3 (Fig. 3). If the treatment was initiated on days 5 and 7 tumour growth was not inhibited. Fifty per cent of mice treated on day 5 did not develop tumours, while the life span of the rest of these mice and all those treated on day 7 was prolonged (Fig. 4).

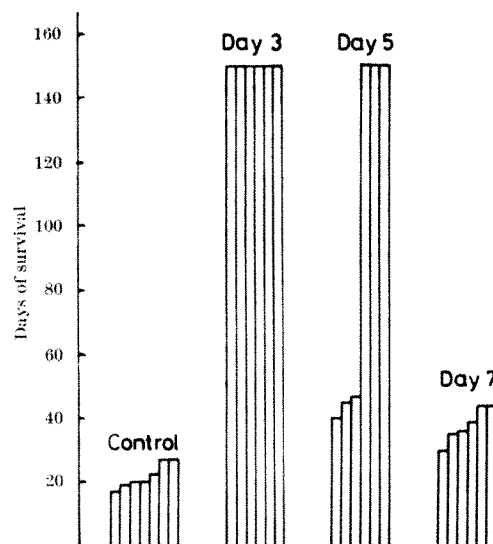


Fig. 4. Survival of mice treated with saline and ricin (injected on days 3, 5 and 7). A second injection was given on day 1 after the first treatment. Each bar represents one animal.

Clearly abrin and ricin not only inhibit the growth of tumour at an early stage but can suppress the tumour completely after it has been developing for several days. Although the medical significance of this finding is not yet clear, our findings add to the biological properties of this class of phytotoxins.

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Selective Inhibition of a Nuclear RNA Polymerase in Cells infected with Frog Virus

Frog virus (FV-3) is one of a group of polyhedral DNA-containing viruses, isolated from normal, tumour and diseased tissues of a variety of amphibia, which replicate within the cytoplasm of infected cells¹. FV-3 multiplies at 12° to 31° C, with a distinctive cytopathic effect, in cell cultures from both poikilothermic and homothermic (including human) vertebrates^{1,2}. The properties of the virion and the characteristics of virus replication have been reviewed by Granoff¹. The earliest demonstrable event in cells infected by FV-3 is a marked depression of cellular RNA synthesis, within 1 h of infection^{1,3}.

We have studied the influence of infection with FV-3 on the nuclear RNA polymerase (nucleoside triphosphate: RNA nucleotidyltransferase, EC 2.7.7.6) activities of baby hamster kidney (BHK 21/C13) (ref. 4) cells, to investigate if the inhibition of the cellular RNA synthesis could be accounted for by a depressed enzymatic activity. BHK cell cultures were grown in stationary Roux-type bottles, each containing 50 ml. of Eagle's basal medium

supplemented with 10 per cent calf serum. When cell monolayers were confluent, the growth medium was discarded and the cultures were infected with 12 ml. of serum-free Eagle's basal medium containing FV-3 in an amount calculated to give a multiplicity of infection of 2 p.f.u. per cell. After 1 h the inoculum was removed and the cell cultures were washed and re-fed with 50 ml. of serum-free maintenance medium. Unless otherwise stated, cell cultures were incubated at 26° C immediately after the infection.

For RNA polymerase assays, at various times (Fig. 1) —reckoned from the beginning of the infection without considering any dead time for virus adsorption—the medium was removed, the monolayer was washed twice with 0.85 per cent NaCl, cells were scraped off the bottles with a rubber policeman and were washed twice with the same solution. After 10 min swelling in 0.01 M Tris buffer (pH 8.0), the cells (approximately 7×10^7 /ml.) were disrupted with a Dounce homogenizer and "nuclear" and "cytoplasmic" fractions were obtained by low speed centrifugation (800g/10 min) at 5° C. Nuclei were washed and resuspended in Tris buffer. The RNA polymerase activity of the nuclear fractions was assayed in a low ionic strength system⁵ containing Mg^{2+} ("5 mM Mg^{2+} " system) and in a high ionic strength^{5,6} system containing Mn^{2+} and ammonium sulphate (" $Mn^{2+}/(NH_4)_2SO_4$ " system).

The rate of RNA synthesis was studied by pulse-labelling cell monolayers with ³H-uridine at various intervals after infection. The medium was removed and the culture bottles were supplied with 12 ml. of serum-free Eagle's basal medium containing 10 μ Ci/ml. of ³H-uridine (specific activity 1.8 Ci/mmol) in the presence of a 20-fold excess of unlabelled thymidine. After 10 min at 26° C, incorporation was stopped by washing the cultures with cold 0.85 per cent NaCl. The cell monolayers were then immediately processed as described to obtain separate nuclear and cytoplasmic fractions. DNA was determined by the method of Burton⁷, and protein by the method of Lowry *et al.*⁸. Uninfected control cell cultures, maintained in the same conditions, were simultaneously processed and tested in each series of experiments.

The results (Fig. 1) demonstrate that the marked depression of RNA synthesis in the nuclei of FV-3 infected cells—which is evident very early in the virus growth cycle—is paralleled by a selective inhibition of RNA polymerase activity detectable in the $Mn^{2+}/(NH_4)_2SO_4$ system; the polymerase activity demonstrable in the 5 mM Mg^{2+} system (not shown in Fig. 1) was practically unaffected. The same results were obtained in reactions at 26° C.

In the cytoplasmic fractions of FV-3 infected BHK cells, studied 1, 3, 8 and 24 h after infection, the RNA polymerase activity was always very low and of the same level as in uninfected control cell cultures. This was evident with the 5 mM Mg^{2+} and the $Mn^{2+}/(NH_4)_2SO_4$ systems, and with the assay system described by Pitkanen *et al.*⁹, both in the presence and absence of detergent (1 per cent 'Triton X'), in the presence or absence of calf thymus DNA primer, and in reactions carried out at either 26° C or at 37° C.

Because the inhibition of host cell macromolecular synthesis by FV-3 occurs at temperatures incompatible with virus replication, or in the presence of protein inhibitors^{1,3}, we have studied the behaviour of RNA polymerase activated at high ionic strength in nuclei of FV-3 infected BHK cells, at either 37° C or 26° C in the presence of 50 μ g/ml. of cycloheximide. Even in these conditions, the nuclei of cells infected by FV-3 have depressed RNA polymerase activity (Table 1), so that, as for the inhibition of host cell macromolecular synthesis, complete virus replication or *de novo* protein synthesis subsequent to infection is not essential for the inhibition of the nuclear polymerase. At the same time, the results suggest that the decreased enzymatic activity cannot be

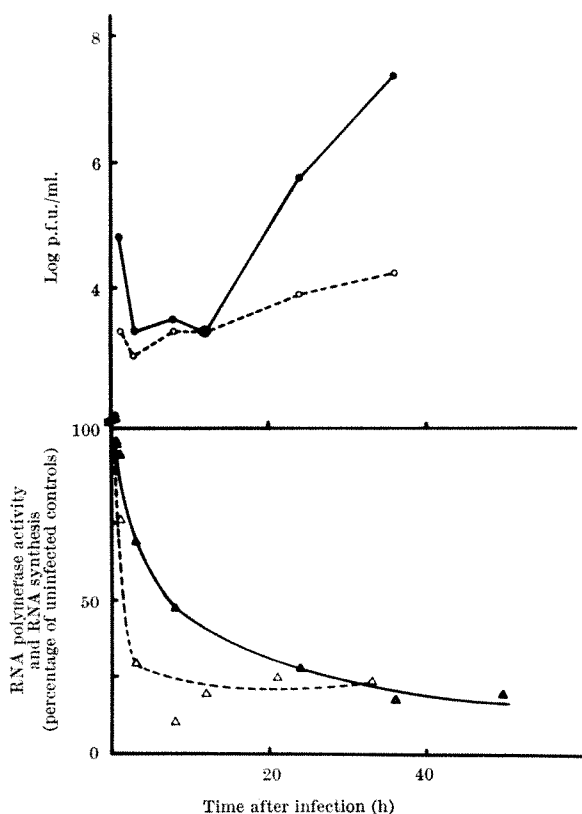


Fig. 1. Top: virus yield in FV-3 infected BHK cell cultures, infected as described. At the times indicated on the abscissa, the fluid and the cells were collected separately. Cells were resuspended in serum-free maintenance medium (in a volume identical to that of the medium fluid) and frozen and thawed three times. The virus present in the medium fluid (\circ --- \circ) and that associated with cells (\bullet — \bullet) were titrated by plaque assay in BHK cells. Bottom: behaviour of high ionic strength activated RNA polymerase and rate of RNA synthesis in the nuclei of FV-3 infected BHK cells. Nuclear fractions were prepared as described. The $Mn^{2+}/(NH_4)_2SO_4$ system (Δ — Δ) contained, in a final volume of 0.5 ml., 50 μ moles of Tris-HCl buffer (pH 7.5), 2 μ moles of $MnCl_2$, 0.035 ml. of ammonium sulphate saturated at room temperature and brought to pH 7.5 with diluted ammonia solution (final concentration in the assay mixture 0.28 M), 0.9 μ mole each of GTP, CTP and UTP, 0.06 μ mole ATP containing 0.3 μ Ci of [³²P]ATP, and 0.1 ml. of nuclear suspension containing about 70 μ g DNA. For both systems, incubation was at 37° C for 10 min. The reaction was stopped by adding 5 ml. of 0.5 N HClO₄. The mixtures were filtered through fibre glass filters (Whatman 'GF/C') which were washed three times with HClO₄, and the radioactivity retained on the filters was measured in a liquid scintillation counter. For the determination of RNA synthesis (Δ --- Δ), "nuclear" fractions were treated with 5 volumes of HClO₄, and the precipitable radioactivity was collected and measured as described.

Table 1. HIGH IONIC STRENGTH ACTIVATED RNA POLYMERASE ACTIVITY IN THE NUCLEI OF CELLS INFECTED WITH FV-3 AND INCUBATED AT NON-PERMISSIVE TEMPERATURE (37° C) OR AT PERMISSIVE TEMPERATURE (28° C) IN THE PRESENCE OF CYCLOHEXIMIDE

Infected	Cell culture conditions Temperature (° C)	Time (h)*	Cycloheximide † (50 µg/ml.)	Polymerase activity ‡ (pmoles of AMP incorporated/mg DNA/10 min)
No	37	3	—	950
No	37	36	—	1,234
Yes	37	3	—	366
Yes	37	16	—	309
Yes	37	36	—	132
No	26	36	—	718
No	26	36	+	580
Yes	26	36	—	124
Yes	26	36	+	140

* After infection or after change of the growth medium with serum-free maintenance medium.

† In the presence of 50 µg/ml. of cycloheximide, protein synthesis in BHK cell cultures maintained at 26° C was inhibited by 92 per cent after 30 min, and by 95 per cent after 1 h, as judged by a further 30 min pulse with 0.5 µCi/ml. of ¹⁴C-protein hydrolysate (*Chlorella*, specific activity 52 mCi/milligram C).

‡ Experimental conditions as in Fig. 1.

ascribed solely to a reduced rate of synthesis of the enzyme, for nuclei from uninfected cells treated with a protein synthesis inhibitor for 36 h show only a moderate decrease in RNA polymerase activity.

The coincidence between the impairment of RNA synthesis and the diminution of the high ionic strength activated RNA polymerase activity strongly suggests that the inhibition of this enzyme is the mechanism underlying the impairment of RNA synthesis in FV-3 infected cells. The $Mn^{2+}/(NH_4)_2SO_4$ -activated RNA polymerase is also selectively inhibited by two toxic substances, α -amanitin¹⁰ and lasiocarpine^{11,12}. Inhibition of this polymerase activity in FV-3 infected cells is accompanied by depression of RNA synthesis, as in animals intoxicated with α -amanitin or lasiocarpine, and this further suggests that the high ionic strength activated RNA polymerase (which may be an enzyme different from that active at low ionic strength)¹³ may be functioning *in vivo*^{10,12,14}.

This seems to be the first example of the selective inhibition of a cellular RNA polymerase activity caused by viral infection. It is unlikely to be an unspecific consequence of viral infection, for in cells infected by vaccinia virus inhibition of RNA synthesis¹⁵ is not accompanied by significant changes in the nuclear RNA polymerase activities¹⁶.

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Fluoroscopic Radiation and Risk of Primary Lung Cancer following Pneumothorax Therapy of Tuberculosis

STEINITZ has reported a remarkable epidemiological study in Israel which indicated that persons with a tuberculosis history, active or arrested, showed a five to ten-fold increase in the risk of subsequent development of primary lung cancer¹. Steinitz was, of course, acutely aware of the excessive risk of lung cancer in cigarette smokers and gave careful attention to this variable. Recently, she has also indicated that there is no reason to believe that persons with a past history of tuberculosis smoked cigarettes more than did those in the general population sample (personal communication). It therefore seems that by some mechanism other than cigarette smoking a record of extensive therapy of tuberculosis implies a high risk of primary lung cancer. The magnitude of excess risk of lung cancer in tuberculous persons is comparable with that for cigarette smoking in non-tuberculous persons. Is tuberculosis *per se* responsible for the observed excessive risk of future primary lung cancer? We think not. We propose that frequent fluoroscopies of the chest, associated with the past extensive use of pneumothorax collapse therapy, is the aetiological factor responsible.

Mackenzie, studying the records of a tuberculosis sanatorium in Nova Scotia, demonstrated a crude twenty-four-fold higher incidence of breast cancer in 271 patients treated with pneumothorax, compared with 510 women who did not receive such collapse therapy, and suggested that radiation may be the cause². Pneumothorax refills had usually been accompanied by a pre- and post-refill fluoroscopic examination. In Mackenzie's series, the average number of fluoroscopies was ~150 per patient treated with pneumothorax. Farber (personal communication) has confirmed that such a frequency of fluoroscopies agrees with his observations for the pre-1950 period in the western USA. From Mackenzie's data, an air dose of ~900 rads, for radiation penetrating 1 mm Al filtration, is indicated to the chest region. This corresponds to 150 fluoroscopies with the typical exposures which were then used. The dose to lung tissue must have been 900 rads or less, depending on the hardness of X-rays used. If those patients not receiving pneumothorax treatment are assumed to have no radiation dose, and if 900 rads are credited for patients averaging 150 fluoroscopies associated with pneumothorax treatment, the overall average lung dose for the Nova Scotia tuberculosis patients is

$$\frac{(900)(271) + (0)(510)}{781}, \text{ or } \sim 310 \text{ rads. Attenuation of radiation}$$

in the lungs would tend to lower this average dose, but the utilization of some fluoroscopies in the non-pneumothorax cases would tend to raise it. The 310 rad average dose is therefore not unreasonable.

Our best estimate, based on Court-Brown and Doll's³ X-ray treated spondylitis and Wanebo's⁴ atomic bomb survivors, is that a dose of ~50 rads produces a number of lung cancers equal to the spontaneous incidence (our unpublished results). Thus 50 rads would constitute a doubling dose for primary lung cancer, and the British and Japanese data, for widely different epidemiological samples, are closely consistent with each other. Thus in a tuberculosis hospital such as that reported by Mackenzie, the average patient would have received 310/50, or 6.2 doubling doses to the lungs. If the spontaneous incidence is added in, the overall lung cancer incidence would be 6.2+1.0, or 7.2 times the expected spontaneous incidence. Such variables as age, sex and cigarette smoking history must, of course, be comparable for the irradiated group and the base population sample.

The Steinitz series of primary lung cancers are for diagnoses made between 1960 and 1963. Because the

patients had had extensive, and in many cases repeated, courses of therapy for tuberculosis, it follows that many of her cases had been treated in the same time period (1940s and early 1950s) as Mackenzie's subjects. It is quite reasonable to believe that practices in tuberculosis therapy would have been similar for the Nova Scotia and Israeli tuberculosis patients. Furthermore, Steinitz indicated that cigarette smoking was not excessive in the tuberculous subjects. The observed five to ten-fold higher risk of primary lung cancer in Israeli tuberculosis cases is therefore consistent with the ~ seven-fold higher expected risk for a fluoroscopic radiation aetiology.

We strongly urge that our hypothesis is accepted only as a guide to valuable studies which can readily be made, and that it should not be considered, at this time, as a plea for reducing essential radiography or fluoroscopy in the management of a serious disease such as pulmonary tuberculosis. Medical practice has already shown concern over unnecessary radiation, and massive dosage reductions have been achieved in fluoroscopic examination.

We present this hypothesis because we believe that worldwide radiation standards are needed with respect to atomic energy development. Neither the Court-Brown and Doll spondylitis series nor the Hiroshima-Nagasaki series is large, and new cases of lung cancer will be added slowly. But records of lung cancer cases can be substantially increased through a worldwide epidemiological study of hospital and follow-up records. Internationally, a very large number of records for tuberculosis patients treated between 1930 and 1950 must be available. Because the latency period for cancer development in such cases would have expired by 1950-70, the relevant lung cancer cases have, in the main, already occurred. A record search plus follow-up should therefore enable fluoroscopic radiation to be evaluated as a possible aetiology of lung cancer in tuberculosis patients. Indeed, in many hospitals the records will be sufficiently accurate to determine the number of fluoroscopies per patient treated, as well as radiation dose per fluoroscopy, and this may even provide a dose-response curve for possible radiation-induction of lung cancer.

Such studies would be far superior, for evaluating this hypothesis, than our comparison of radiation dose in Nova Scotia with lung cancer incidence in Israel. The proposed studies would have internal controls in two important ways. First, within a particular study, the lung cancer incidence rate as a function of the number of fluoroscopies can be determined for non-smokers, and a similar comparison can be made for cigarette smokers. Second, the studies in a particular region in a single country would provide controls, similar to the irradiated persons, in all variables other than fluoroscopic radiation. Finally, while pharmacological agents should be considered, this factor would have been of little consequence for therapy before 1950. Indeed, a preliminary follow-up study by Hammond and co-workers provided no evidence for excessive cancer in tuberculosis patients treated with isoniazid, one of the chemicals reported to produce neoplasia in mice⁵. It is also of interest that Hammond reported no excessive cigarette smoking in 311 tuberculous subjects compared with non-tuberculous persons.

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Synthetic Sweetener Consumption and Bladder Cancer Trends in the United States

RECENT experimental evidence has demonstrated that high doses of synthetic sweeteners cause bladder cancer in rodents. In rats, the oral administration of a cyclamate-saccharine mixture alone or combined with cyclohexylamine, a metabolite of cyclamate, induced a high frequency of bladder carcinoma¹. These tumours also developed in mice after bladder implantation of cholesterol-cyclamate or cholesterol-saccharine pellets^{1,2}. These studies prompted the Department of Health, Education and Welfare to restrict the general use of cyclamates and to undertake further laboratory studies on the toxicity of synthetic sweeteners. As a first step in evaluating whether synthetic sweeteners are carcinogenic in man, we have examined the consumption patterns of these agents and the trends for bladder cancer in the United States.

A dramatic increase in the consumption of artificial sweeteners began in the US in 1962, chiefly in the form of a 10:1 cyclamate-saccharine mixture (Fig. 1). The consumption of cyclamates rose from 2.7 million pounds in 1962 to 19.7 in 1969. Although the exposure to cyclohexylamine is unknown, it is present in some cyclamate-sweetened food as a result of processing, and in some persons cyclamate is metabolized to cyclohexylamine³.

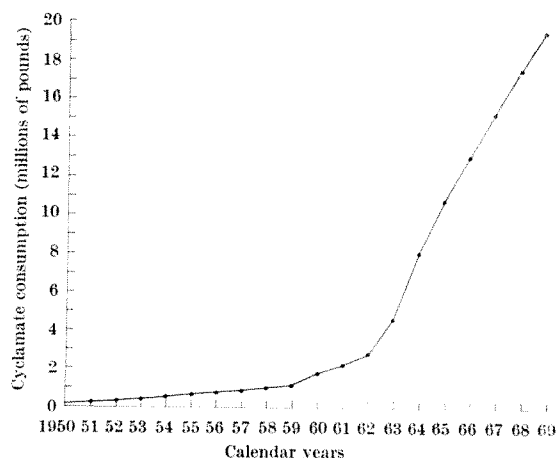


Fig. 1. Cyclamate consumption in the US, by calendar year, 1950-1969 (unpublished data, US Department of Agriculture).

A wide variety of foods and drugs have contained synthetic sweeteners; about 70 per cent of all cyclamate-saccharine mixtures were consumed as soft drinks. While per capita consumption of these beverages was fairly uniform throughout all age groups, the greatest exposure occurred in persons between 16 and 19 years of age. In that age group, average daily cyclamate consumption from soft drinks was 1 mg/kg of body weight, and maximum intake about 50 mg/kg².

Death rates for bladder cancer were calculated for the US in 1950-1967, from mortality data on computer tapes representing death certificates kept at the National Center for Vital Statistics and from population data derived from published census statistics. Although there are differences in mortality by race and by sex, no clear-cut break in either age specific or age adjusted rates has occurred since the mass introduction of synthetic sweeteners in 1962 (Fig. 2). Because a person with bladder cancer may be cured or may survive for a long time, mortality is less sensitive than incidence in detecting a change in frequency. Study of age specific and age adjusted incidence rates for bladder cancer in Connecticut^{3,4}, supplemented by unpublished tabulations for 1965-1967, shows that recent trends are consistent with

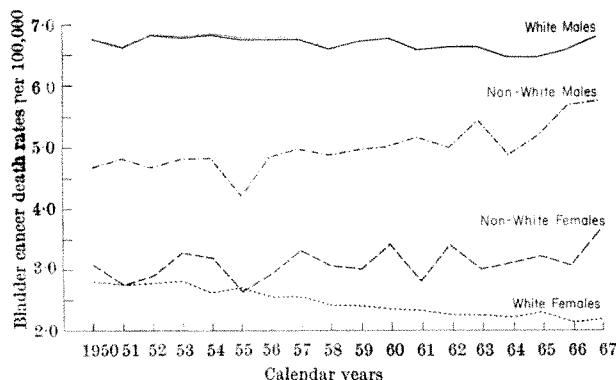


Fig. 2. Bladder cancer death rates in the US, age adjusted, by race, sex, and calendar year, 1950-1967.

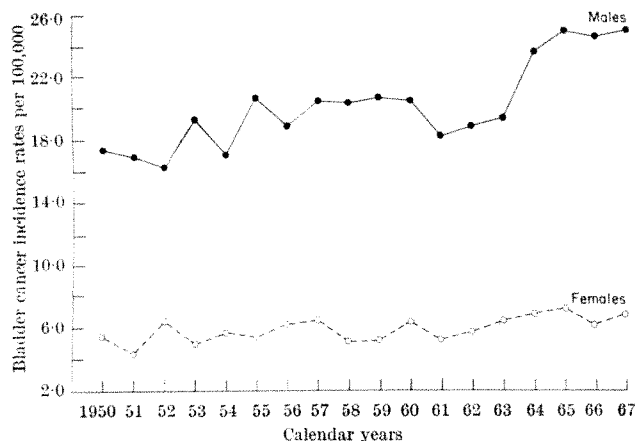


Fig. 3. Bladder cancer incidence rates in Connecticut, age adjusted, by sex and calendar year, 1950-1967. (Data for 1966 and 1967 are estimates corrected for delayed reporting.)

the slow, long-term rate of increase (Fig. 3). Incidence figures from New York state⁵, supplemented by unpublished data for 1961-1967, confirm this trend. Many epidemiological features of bladder cancer, such as the increasing incidence, male predominance and geographic variation, may be attributed to patterns of cigarette smoking, which increases the risk of this neoplasm about three-fold^{6,7}.

Although the mass introduction of synthetic sweeteners into the general diet has not influenced bladder cancer rates in the United States up to 1967, an oncogenic effect may have gone undetected if the dose were too low or if the observation period were too short. The dose per body weight which produced tumours in experimental animals was fifty times higher than the maximum amount ingested by man^{1,2}. Furthermore, only six years of observation have passed since the sharp increase in consumption of synthetic sweeteners, while the latent period averages twenty years for tumours induced by occupational exposure to aniline dyes, betanaphthylamine and other chemicals⁸.

The carcinogenic potential of cyclamate or cyclohexylamine is suggested not only by the induction of bladder tumours in rodents, but also by chromosome breaks *in vivo* in the spermatogonia and bone-marrow cells of rats⁹ and by chromosome breaks *in vitro* in human cells⁹. While detailed studies of bladder cancer trends in the general population should be continued over the next two or three decades, the long-term effects of cyclamates and other sweeteners may be detected more readily by monitoring special groups of heavily exposed persons.

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Control of Potato Gangrene and Skin Spot Diseases by Fumigation of Tubers with Sec-butylamine

FUNGAL diseases of potato tubers can cause considerable losses in the United Kingdom every year. At present in Scotland the most serious is gangrene, a rot of tuber flesh caused by *Phoma exigua* var. *foveata*. Skin spot, caused by *Oospora pustulans*, is also important, but in this case the organism does not rot tuber flesh: small pustules appear on the surface and, more importantly, eyes may be killed and sprouting affected.

These diseases have proved difficult to control chemically, and until very recently this has only been achieved by disinfecting tubers in solutions of organo-mercury compounds¹. Mercury treatment has been adopted commercially, but it suffers from several practical difficulties, and although used for many years has made only limited impact on the seed trade. Experimental use of the systemic benzimidazole fungicides is giving promising results, but these still have to be applied to tubers as dusts or in dipping solutions².

Gaseous treatment of tubers has not often been investigated, although formaldehyde gas has been tried unsuccessfully. Nevertheless, fumigation has attractive possibilities especially because gases could be introduced into bulks of stored tubers fairly easily. We tested several gases against pure cultures of gangrene and skin spot fungi but substances that seemed promising fungicides proved phytotoxic to tubers. Although sec-butylamine (2-aminobutane) had only a limited effect on growth of pure cultures, we decided to see whether it would control natural tuber infections. This substance and its salts have been shown to be actively fungistatic by Eckert and Kolbezen³, who used them chiefly against fungal rots of citrus fruits. Sec-butylamine is a colourless liquid with an ammoniacal odour, easily vaporized (boiling point 63° C), miscible with water and most organic solvents. It is a moderately toxic substance, the toxicity being due primarily to its strong alkalinity, but effects are minimized by both dilution and neutralization.

In the first tests 38 kg of freshly lifted naturally infected tubers of several cultivars were fumigated with different doses of gaseous sec-butylamine in a 400 l. steel fumigation chamber fitted with an external circulatory system to assist application and distribution of the gas. The potatoes were exposed to the gas for about 2 h during which most of it was absorbed.

Table 1. EFFECT OF TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF GANGRENE

Cultivar	Dosage (mg/kg) <i>sec</i> -butylamine	No. of tubers examined	Per cent gangrene
Majestic, stock 1	85	229	6.1
	140	227	4.8
	Nil	195	33.3
Majestic, stock 2	140	172	4.7
	Nil	171	18.1
King Edward	140	278	2.5
	350	288	4.2
	Nil	259	25.9

Table 2. EFFECT OF TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF SKIN SPOT (CULTIVAR KING EDWARD)

Cultivar	Dose (mg/kg)	Percentage of tubers affected in each category				Skin cover score*			
		Free	Some	All	None	Trace	Slight	Moderate	Severe
Stock 1	70	84	16	0	68	30	2	0	0
	120	76	24	0	66	26	8	0	0
	Nil	24	48	28	10	28	58	4	0
Stock 2	140	98	2	0	92	6	2	0	0
	350	100	0	0	98	2	0	0	0
	Nil	66	24	10	30	36	20	14	0

* Trace, 1-10 pustules per tuber; slight, up to 1/10 of tuber surface covered; moderate, between 1/10 and 1/4 surface covered; severe, more than 1/4 surface covered.

The treated and untreated tubers were placed in wooden sprouting trays and stored over winter. All the tubers were examined periodically until the end of March for the appearance of gangrene lesions, and skin spot was assessed at the end of the storage period by Boyd's method⁴ using fifty tubers. Results of some of the experiments are shown in Tables 1 and 2. Because the treatments showed such promising control of both diseases with no evidence of tuber phytotoxicity we built a large experimental fumigation chamber to hold 5 tons of tubers, fitted with a forced draught gas recirculation system incorporating an apparatus capable of vaporizing the required amount of *sec*-butylamine in approximately 40 min. Tubers were fumigated within 48 h of lifting with a dose of 200 mg/kg. Analyses of gas samples drawn through tubes ending at different positions in the bulk showed that even distribution of the gas throughout the air spaces between tubers was only obtained slowly. During the application considerable condensation and adsorption took place on the lower layers of tubers, and this was apparently followed by processes such as evaporation, desorption and further adsorption throughout the bulk when recirculation of the mixture of air and gas was continued after all the fumigant was applied. Residue analyses on samples taken from various positions in the bulk indicated that good distribution of the fumigant on tubers had been achieved after recirculation for a further 2 h. After fumigation 1/2 ton bulks made up of tubers taken from different positions in the chamber were stored under straw in a shed until February, when they were sorted and assessed for rot diseases. The healthy material was then boxed. Further disease assessments were made in early April, and the visual diagnoses checked by isolation of fungi from representative tuber samples. For controls 1/2 ton untreated lots from the same consignment were stored similarly. Results of four experiments are shown in Tables 3 and 4.

Table 3. EFFECT OF BULK TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF GANGRENE

Cultivar	Treatment	No. of tubers examined	Per cent gangrene
Majestic	200 mg/kg	1,328	0.2
	Nil	1,376	4.1
Redskin	200 mg/kg	567	0.7
	Nil	552	88.6

Table 4. EFFECT OF BULK TREATMENT WITH *sec*-BUTYLAMINE ON THE INCIDENCE OF SKIN SPOT

Cultivar	Treatment	Percentage of tubers affected in each category				Skin cover score			
		Free	Some	All	None	Trace	Slight	Moderate	Severe
King Edward	200 mg/kg	100	0	0	100	0	0	0	0
	Nil	13	56	31	0	20	70	9	1
Majestic	200 mg/kg	100	0	0	98	2	0	0	0
	Nil	14	76	10	2	32	46	18	2

As well as a visual examination for skin spot, eye plug samples from the King Edward stock were incubated in moist chambers and examined microscopically for the development of *O. pustulans*⁵, which was not detected. The tuber diseases dry rot (caused by *Fusarium caeruleum*) and blight (caused by *Phytophthora infestans*) were not controlled, but the effect of fumigation with *sec*-butylamine on other diseases has not yet been investigated.

Sec-butylamine in a dose of 200 mg/kg had no phytotoxic effect on undamaged tubers although badly skinned areas, especially on immature tubers, could be discoloured. Extensive skin damage and complete death of eyes were caused by a dose between 1,000 and 5,000 mg/kg. Preliminary results of field trials showed that treatment of tubers with *sec*-butylamine at 200 mg/kg had no adverse effect on plant growth or yield. Experiments are continuing on various aspects of the treatment including determination of residues in treated tubers and in crops grown from treated tubers. Studies are also being made on the physical processes operating in fumigating bulks of tubers with *sec*-butylamine. United Kingdom and Irish patents have been applied for.

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Phenolase Inhibitor from Cultures of *Penicillium expansum* which may Play a Part in Fruit Rotting

PREVIOUS work in this laboratory¹ has shown that the light colour of apples infected with *Penicillium expansum* ("blue-mould") is due to the inhibition by the fungus of the fruit's normal enzymatic browning system. A positive feedback system operates whereby the fungal pectolytic enzymes modify the apple's phenolics to yield inhibitors of apple phenolase (*o*-diphenol oxidase) and the associated biochemical defence system. But the problems of the initial establishment of fungal growth remain and I wish to report evidence that the fungus secretes its own phenolase inhibitor until it is sufficiently established to allow the second, major positive feedback system to take over.

Cultures of *P. expansum* were grown as mats on a yeast-malt broth² or on Czapek-Dox medium at 26° C. The culture medium was sampled aseptically at suitable intervals and tested for its ability to inhibit the oxidation of catechol by a phenolase preparation from apple chloroplasts³. In yeast-malt medium the concentration of inhibitor was maximum between 36 and 48 h of growth and had diminished to zero after 96 h when the mycelial mat had begun to sporulate. By contrast, cultures on Czapek-Dox medium grew more weakly and formed less inhibitor even after incubation for 14-16 days.

The biochemical properties of this phenolase inhibitor, which I call "expansin", were investigated using the original culture medium, or an ethereal extract prepared by extraction of the broth with diethyl ether followed by a single wash with a saturated solution of NaHCO₃. Dixon's method showed that "expansin" competitively inhibited the oxidation of chlorogenic acid, catechin and catechol by particulate phenolase preparations from apples and tobacco leaf, whereas it had no effect on a laccase (*p*-diphenol oxidase) preparation from *Glomerella*

*cingulata*⁵. The degree of inhibition increased by up to 50 per cent if the extract was pre-incubated with the enzyme for longer than 40 min before addition of substrate. A similar effect of time of pre-incubation was observed by Harel *et al.*⁶ for a phenolase inhibitor extracted from the mycelium of *Dactylium dendroides*⁷.

Chemical investigations of expansin were hampered by its instability in aqueous solution and the very low concentrations present in the culture medium. Inhibitory activity was destroyed by boiling or by alkalis stronger than NaHCO_3 . The crude ethereal extract was examined by chromatography and electrophoresis on thin-layers. The bands were scraped from the dried thin-layer chromatography (TLC) plates into tubes containing buffer (0.1 M phosphate, pH 5.0) and phenolase preparation in a final volume of 5 ml. Substrate (10 μmoles of catechol + 50 μmoles of glycine) was added 40 min later and the tubes were incubated, with shaking, at 30°C. If expansin was present the reaction mixture remained colourless while control tubes turned red-brown. Expansin had R_f values of 45–50 in 2 per cent (v/v) acetic acid and 90–95 in a mixture of benzene, acetic acid, water (125:72:3) or a mixture of *n*-butanol, acetic acid and water (12:3:5). These zones did not correspond with areas of fluorescence on the TLC plates, nor did they react when tested with various chromogenic spray reagents, probably because of the low concentration present. Expansin is probably neutral, for it did not migrate appreciably in an electric field (20 V/cm, pH 2.0) nor was it absorbed by ion-exchange resins. Chromatography on columns (42 x 2 cm) of 'Sephadex G-15' with water as the eluant suggested that expansin had a relatively low molecular weight. The ultraviolet spectra of the fractions which contained inhibitor showed maximum absorption at 280–290 nm. Attempts to purify further these fractions from the 'Sephadex' were unsuccessful. Comparative tests with several known metabolites from *Penicillium* sp.⁸, such as patulin, gentisaldehyde, gentisic acid, 6-methyl-salicylic acid and *p*-hydroxybenzoic acid confirmed that these compounds were devoid of inhibitor activity at concentrations up to 0.01 M.

Harel and his collaborators⁶ have reported that the fungus *D. dendroides* secretes a small peptide which inhibits apple phenolase⁶ and galactose oxidase⁷, both enzymes which contain copper, by reacting with the prosthetic group. But the evidence is that expansin is not a peptide and, furthermore, it acts by competition with the substrate.

The findings are interesting from the point of view of the biochemistry of the relationship between host and parasite, and could explain the mechanism whereby *P. expansum* overcomes the apple's defence mechanisms during the earliest stages of infection and mycelial growth. If expansin is formed *in vivo* it could suppress enzymatic browning until the fungal extracellular pectolytic enzymes have developed and modified the phenolics of the apple to yield phenolase inhibitors such as *p*-coumaric acid and ferulic acid¹.

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Initiation of Flowering on First Year Cuttings of *Metasequoia glyptostroboides* Hu and Cheng

AFTER its discovery in the interior of China at latitude 30° N in 1941, *Metasequoia glyptostroboides* was introduced to Britain in 1948, and has been propagated by cuttings and established in botanical gardens and parks throughout the country. Although there are now specimens up to 16 m high, flowering has been confined to occasional female cones, which in the absence of pollen have not produced viable seeds¹. Male cones do not seem to have been recorded here² although both seedlings and cuttings growing in the Crimea at 45° N have produced them after an interval of 7 yr³. Flowers of both sexes have been induced in Japan by strangulation⁴ and by treatment with gibberellic acid^{5–7}.

Both male and female cones have now been initiated on 1 yr old cuttings grown in a greenhouse in Edinburgh. In September 1968, shoots with vegetative buds were taken from a tree aged about 14 yr from planting (probably itself a cutting), treated with 'Seradix' No. 2 rooting powder and placed under mist. The rooted cuttings were potted in December 1968, and six were kept in partially controlled greenhouse conditions for observation. The male cones were first seen on November 22, 1969, partially emerging from buds on one plant, and male initials have since been found on two more plants. Eleven male cones have been identified, together with twenty-eight possible flower buds, all occupying terminal positions on branchlets which are usually budless and deciduous, both the simple and the branched type (Fig. 1). In plants growing outside, such shoots normally cease development and fall off by late November, but they were retained up to 3 months longer on the plants in the greenhouse. Most of the fertile branchlets eventually abscised, but two were retained for 6 weeks in a cold room at 6°C. These chilled cones emerged fully (Fig. 2), and have shed pollen freely. Five female cones emerged at the same time, some from lateral buds on the main stem, and some in a similar position to the male (Fig. 3). These have now been self-pollinated, and have begun to enlarge.

It seems likely that this early initiation of flowering may have been encouraged by the long period in warm conditions. The plants were not chilled during the winter of 1968–69, and they flushed in late March 1969 and rapidly completed extension of their terminal shoots. During September and October, many lateral shoots extended, providing the loci at which flowers could be initiated.

Although these investigations are at a very early stage, they are interesting for several reasons. Production of



Fig. 1. *Metasequoia* cutting No. 6 on December 1, 1969. Two male cones can be seen partially emerging without chilling (bottom of picture). Above them, two shorter fertile branchlets can be seen, whose buds emerged after chilling (Fig. 2).



Fig. 2. The same cutting as in Fig. 1. March 6, 1970, two days before the two male cones began to shed pollen. The length of a cone is about 4 mm, and of a leafless "stalk" about 3 mm. Each cone scale bears two or three abaxial pollen sacs.

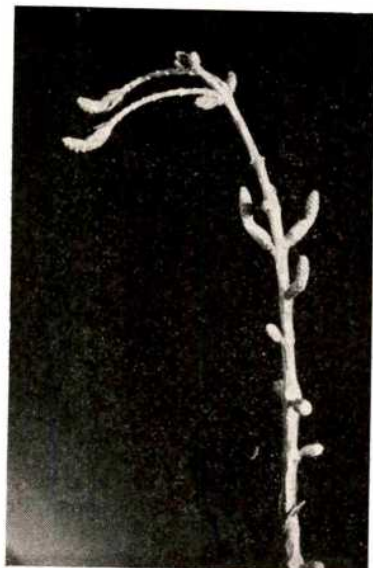


Fig. 3. The top of the same cutting as in Fig. 1, on March 6, 1970. There are two female cones at the ends of branchlets, and three in lateral positions on the main stem. There are vegetative buds below and near the apex. The length of female cones is 4–6 mm on leafy "stalks" which subsequently extended rapidly.

pollen may make it possible to produce fertile seed for the first time in Britain, and also to cross selected clones with one another to raise improved planting stock. Such genetical work is always facilitated when flowering can be controlled, but the study of the reproductive physiology of forest trees has been hampered by the technical problems of experimenting with large plants. *Metasequoia* cuttings such as these, which were only 30 cm high, clonal, and growing well in 5 inch pots, would provide almost ideal material for growth-room investigations into the effects of day length, temperature, hormones or other treatments on flower initiation. Similarly, studies of flower bud development, pollination and seed production would be possible in controlled environments, and we may be able to deal with the juvenile,

purely vegetative phase in coniferous trees, for seedlings of this species seem to be capable of producing female cones before reaching an unmanageable size³.

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Biological Control of Decay Fungi in Wood by Competition for Non-structural Carbohydrates

HYPHA of fungi that decay wood spread rapidly through their host substrate before any significant loss of weight is detectable¹. Indeed, the rate of advance of *Fomes annosus* over or through red pine was identical with its growth rate over malt-agar plates² even though fungi that decay wood develop more slowly on cellulose than on simple sugars³. Glucose stimulates growth over wood⁴, and laboratory cultures provided with a choice of cellulose or a simple carbon source preferentially utilize the latter^{5,6}, for enzymes that hydrolyse cellulose are generally only produced when growth is restricted⁷. These data suggest, as expected, that fungi initially colonize wood chiefly by utilizing non-structural carbohydrates. Such materials would include simple sugars which have been identified in the sapwood of both hardwoods⁸ and softwoods⁹ and which in the latter account for 0.03 to 0.8 per cent of the dry weight; figures for the inner bark are, of course, considerably higher. Polysaccharides such as starch may also be utilized rapidly: ray cells often provide entrance for invading hyphae and starch reserves of these cells were quickly exhausted, for example, during early decay of sweetgum sapwood by *Poria monticola*¹⁰. Traces of polysaccharides grouped with holo-cellulose may also be readily available and thus utilized early in the decay process¹¹.

During natural decay of wood, many ecological factors determine the course and succession of fungal colonization¹², but in conditions that are favourable for many wood-inhabiting fungi, the success of a fungus as a primary wood saprophyte may largely depend on its ability to spread rapidly through wood by utilizing non-structural carbohydrates. A guide to this ability might be obtained by using similar conditions to assess the rate of development of the wood-inhabiting fungus on simple sugars. *Trichoderma viride*, for example, often colonizes wood, and although it can decompose cellulose, its ability to utilize structural polysaccharides of wood is very limited^{13–15}; it also grows very rapidly over malt-agar plates at 20–30° C (ref. 16). *T. viride* may retard the development of other fungi by producing the antibiotics gliotoxin or viridin, although attempts to demonstrate this with plate cultures have been unsuccessful¹⁶. Tests with liquid cultures indicated that these antibiotics may not be produced by *T. viride* but by the closely related *Gliocladium*¹⁷. Perhaps *T. viride* retards colonization by other fungi by rapidly removing the non-structural carbohydrates of wood which seem to be necessary for rapid hyphal progression. A similar conclusion explained the antagonism of soil bacteria towards *Fusarium oxysporum*

Table 1. PERCENTAGE LOSS OF WEIGHT OF OVEN-DRIED BLOCKS OF BIRCH SAPWOOD DURING 4 MONTHS INCUBATION WITH ONE OR TWO FUNGI AT 27° C

	<i>T. viride</i>	<i>G. viride</i>	C134	None
<i>P. adustus</i>	16	29	48, 4*	37
<i>P. hirsutus</i>	1	11	—	64
<i>P. versicolor</i>	3	33	—	82
None	2	2	4	—

Blocks (2 × 2 × 2 cm) containing 45 per cent moisture were placed on the surface of soil containing 40 per cent moisture. Inocula were grown on birch-wood slips on 2 per cent malt-agar; two slips were then placed underneath each wood block. When two fungi were used they were allowed to develop on separate slips. Values are an average of four replicates.

* Inoculation with *P. adustus* delayed for 1 month.

f. sp. *cubense*, in which inhibition of fungal growth was not due to antibiotics and only occurred when nutrient concentrations were low¹⁸.

To test this hypothesis we evaluated fungal interaction in birch (*Betula papyrifera*). We used common isolates from decaying birch wood including (together with their growth rates over malt-agar plates at 27° C) *Polyporus adustus* (17 mm per day), *Polyporus hirsutus* (13 mm per day) and *Polyporus versicolor* (13 mm per day), all of which cause a typical white rot; C134 (2 mm per day), an unidentified, non-sporulating, apparently non-destructive fungus, and *T. viride* (27 mm per day). *Glaciadium viride* (22 mm per day) isolated from decaying aspen wood was also used. Clear zones of inhibited growth due to the production of antibiotics could not be detected in malt-agar plate cultures of any of the three species of *Polyporus* paired with either C134, *T. viride* or *G. viride*; the basidiomycetes often overgrew the other fungus.

The same pairs of fungi were used to inoculate blocks of birch sapwood in a soil-block test; results are shown in Table 1. *T. viride*, the fastest growing fungus on malt-agar, provided the best protection from decay by the basidiomycetes. The possible production of antibiotics by *G. viride* seemed to be of minor ecological importance and its comparative ability to prevent decay was more closely correlated with its rate of growth over malt-agar. C134 provided no protection and even increased decay by *P. adustus*, demonstrating growth synergism¹⁹, unless the former was developing alone as the primary saprophyte. Of the basidiomycetes, *P. adustus* caused the least loss of weight when used alone but was the most successful basidiomycete in these competitive soil-block trials and was also the fastest growing basidiomycete over malt-agar. In all experiments, blocks that had lost a little weight were sound and unstained after incubation. Rotted blocks containing two fungi were decayed only on the side inhabited by the basidiomycete.

Results of field trials involving random infection of birch logs further supported the hypothesis. Table 2 shows that immediate inoculation with *T. viride* afforded excellent protection to the logs when stored for 7 months, even when followed by attempts to infect the logs with *P. adustus* 2 weeks after inoculation. By contrast, the slower growing C134 was totally inadequate. If inoculation

with *T. viride* was delayed for 4 days other fungi, generally those which grow most rapidly over malt-agar plates at ambient temperatures, rapidly infiltrated the wood, demonstrating the speed of this initial process of colonization. Similarly, if *P. adustus* was used for the immediate inoculation it colonized the logs almost to the total exclusion of other fungi, but if inoculation was delayed for 2 weeks any advantage was lost.

The results are of practical significance in attempts to protect wood from decay. In addition, the permeability of wood to preservatives is considerably enhanced after colonization by *T. viride*²⁰. Although *T. viride* seems to approach the practical ideal of a scavenging cellulolytic fungus which can rapidly remove non-structural carbohydrates from wood without substantially altering its mechanical properties or physical appearance, comprehensive screening programmes will doubtless reveal better organisms for this purpose, particularly in different environmental conditions.

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Export of Photosynthate Affected when Leaves are Pretreated with Growth Substances

SEVERAL investigators have shown that cytokinins are strong mobilizing agents¹⁻³. For example, a spot of cytokinin on a leaf can attract such substances as amino-acids from adjacent untreated tissues, and treatment of fruits^{4,5}, shoot tips⁶, or expanded leaves⁷ increases movement of photosynthate into the treated area. Gibberellic acid also enhances movement of assimilates into the treated area, but the growth retardant CCC ((2-chloro-ethyl)-trimethylammonium chloride) causes a decrease in the mobilizing power of the treated area^{4,6}. We are not aware, however, that leaves on intact plants have ever been pretreated with growth regulators to study the effect on the export of photosynthate from the treated leaves.

Rootings of *Vitis vinifera* L. 'Thompson Seedless' were grown in 'Sponge Rok' in 1 gallon containers. Hoagland No. 1 solution at half strength was used to irrigate the plants⁸, which were used when the shoots were 12-14 inches long. In each experiment, young, fully expanded leaves which were exporting photosynthate upwards in

Table 2. PRINCIPAL FUNGAL ISOLATES FROM BIRCH LOGS STORED IN OTTAWA

Inoculum history	<i>T. viride</i>	C134	<i>P. adustus</i>	<i>P. versicolor</i>	Unidentified basidiomycete B344
<i>T. viride</i> immediately	202	0	6	6	0
<i>T. viride</i> after 1 day	116	0	10	5	0
<i>T. viride</i> after 4 days	88	0	46	14	0
<i>T. viride</i> after 1 week	49	0	42	36	0
<i>T. viride</i> immediately and <i>P. adustus</i> after 2 weeks	241	0	1	0	0
C134 immediately and <i>P. adustus</i> after 2 weeks	4	4	101	7	38
<i>P. adustus</i> immediately	7	0	212	0	0
<i>P. adustus</i> after 2 weeks	56	0	107	5	29
Control (sterile water immediately)	17	0	109	19	43

The fresh sound birch logs were 120 cm long and 15-25 cm in diameter. A disk, 8 cm thick, was sawn from each end at the start of the experiment in early May. After inoculation by spraying the ends with a mycelial and/or spore suspension the ends were covered with polythene for 3 days. After 7 months a fitch 8 cm thick was sawn from the centre of each log and a row of six isolations attempted every 15-20 cm along each side by placing the removed chip on 2 per cent malt-agar.

* *P. hirsutus* was only found in the control (30 isolates).

the shoot were dipped momentarily into a solution containing a growth regulator. After 3 h the treated leaves were exposed for 30 min to $^{14}\text{CO}_2$ from labelled barium carbonate (0.5 mg) with a specific activity of 0.05 mc./mg. The label was applied to the leaves according to the

method of Hale and Weaver⁹. Six hours after the beginning of exposure the shoots were collected, mounted and autoradiographed according to the method of Yamaguchi and Crafts¹⁰. The X-ray film was exposed to the mounts for 7 days. Three replicate shoots were used in each treatment.

In one experiment the leaves were dipped in benzyladenine at 4,000 p.p.m. Corresponding leaves on the control shoots were exposed to $^{14}\text{CO}_2$ without prior treatment with benzyladenine. In the controls, the photosynthate was exported from the leaves and, after entering the stem, moved acropetally. Only a small amount of assimilate was exported from the treated leaves. As a result of treatment with benzyladenine, the fully expanded leaves, which normally export photosynthate to the shoot tips, had become strong "sinks" for assimilate, so that little export occurred. When 1,000 p.p.m. of gibberellic acid was used instead of benzyladenine, results were similar.

When 4,000 p.p.m. of the plant growth retardant (2-chloroethyl)trimethylammonium chloride (CCC) was used, photosynthate moved out of the treated leaves at the same rate as from the untreated leaves. In both cases, movement in the stem was upward. The export of photosynthate out of the leaves treated with CCC is in agreement with earlier findings that this compound tends to counteract the mobilizing power of the leaves as a "sink"⁶. Growth regulators have been shown to have no marked effect on CO_2 fixation in plants¹¹, so this factor can be eliminated in the explanation of our results.

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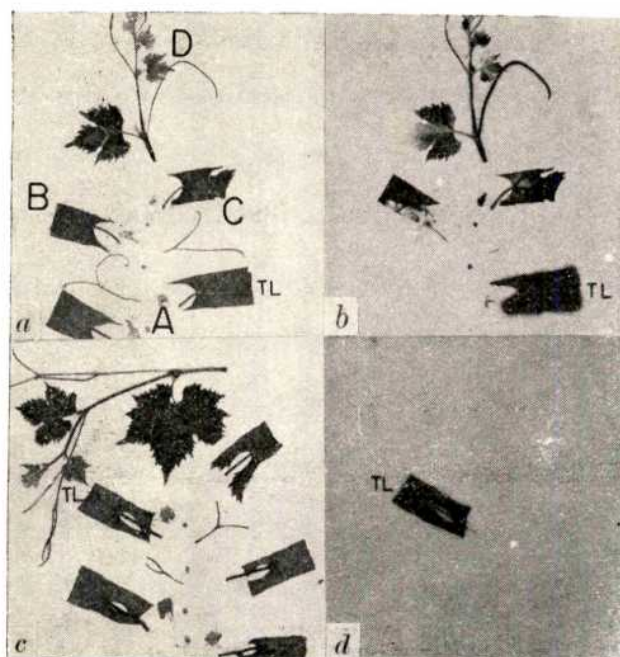


Fig. 1. (a) Mounted specimen and (b) autoradiograph of untreated shoot in which upper leaf was exposed to labelled carbon dioxide. A, Section of internode; B, portion of leaf and petiole; C, tendril; D, shoot tip; TL, treated leaf. The dark areas of the autoradiograph indicate the presence of carbon-14, the darker the area the greater the concentration of the isotope. Translocation of carbon-14 was upward. (c) Mounted specimen and (d) autoradiograph of shoot of which upper leaf was treated with benzyladenine before exposure to labelled carbon dioxide. Note that there was very little export of labelled carbon dioxide from the treated leaf.

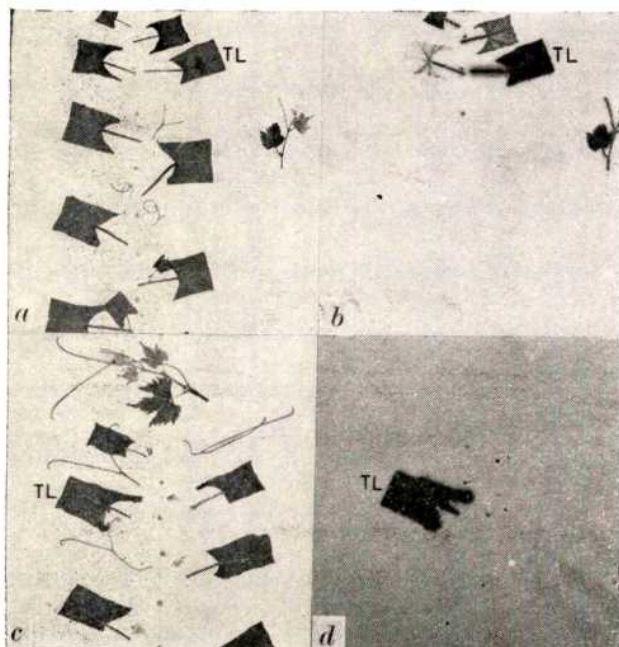
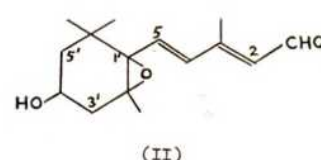
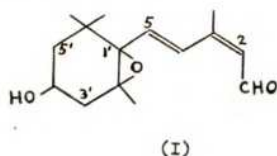


Fig. 2. (a) Mounted specimen and (b) autoradiograph of shoot of which upper leaf was treated with CCC ((2-chloroethyl)trimethylammonium chloride) before it was exposed to labelled carbon dioxide. TL, Treated leaf. Note that translocation of carbon-14 was upward. (c) Mounted specimen and (d) autoradiograph of shoot of which upper leaf was treated with gibberellic acid before it was exposed to labelled carbon dioxide. Note that there was very little export of labelled carbon dioxide from the treated leaf.

Xanthoxin, a New Naturally Occurring Plant Growth Inhibitor

We have extracted a neutral growth inhibitor from the seedlings of dwarf bean (*Phaseolus vulgaris* cultivar 'Canadian Wonder') and wheat (*Triticum vulgare* cultivar 'Hybrid' 46). Comparison of chemical, chromatographic and bioassay data has shown it to be a mixture of two isomeric compounds which we have characterized as products of the photo-oxidation of violaxanthin¹, a common plant xanthophyll. They are the highly active 5-(1',2'-epoxy-4'-hydroxy-2',6',6'-trimethyl-1'-cyclohexyl) 3-methyl-*cis,trans*-2,4-pentadienal (I) and the less active *trans,trans* isomer (II). The name xanthoxin is now proposed for the inhibitor, the activity of which in the *cis,trans* configuration is comparable with the known naturally occurring inhibitor abscisic acid (ABA) in several tests.



Bean seedlings were grown on 'Vermiculite' in a 16 h photoperiod (800 lumens/foot² from natural daylight fluorescent tubes) at 25° C (night temperature 24° C). Eight day old shoots (250 g fresh weight) were frozen in ether (1.5 l.) at -20° C, crushed and maintained at 3° C in darkness for a 24 h extraction period. All subsequent operations were carried out in a shaded laboratory. The ether extract was decanted from the tissue and shaken with 2 per cent sodium bicarbonate solution (twice) and water to remove acids. The ether was removed at reduced pressure and the extract was redissolved in light petroleum (60°-80° C). This solution was partitioned with an equal volume of 80 per cent aqueous methanol, which was then adjusted to 50 per cent by the addition of water, and the xanthophylls were largely removed by further partitioning with light petroleum. The methanol was distilled from the extract, and, after the addition of sodium sulphate, the aqueous solution was re-extracted with ether.

Further purification of this ether extract was carried out on a column (18 mm bore) containing 100 mesh silicic acid powder (20 g), using a mixture of benzene and ethyl acetate (2:1) as solvent and collecting 10 ml. fractions. Each of these forty fractions was assayed for plant growth inhibitors in the wheat coleoptile section test² (using 2 ml. aliquots) and the cress seed germination test² (using 0.2 ml. aliquots). The results of these tests (Fig. 1) showed that the principal inhibitor was present in fractions 21-27. These fractions were combined and two aliquots (4.0 and 0.4 ml.) were streaked onto a chromatoplate ('Kieselgel GF 254', 20 × 20 cm, 0.2 mm thick) with a mixture of the violaxanthin photoproducts (I) and (II) as a reference. After developing three times in light petroleum-acetone (3:1), 20 equal 7.5 mm strips of silica gel were removed from each of the separated extracts, and eluted with methanol (1 ml.). The eluates from the 4 ml. aliquot were assayed in the coleoptile section test, and those from the smaller aliquot in the germination test. In both of these tests there was a single zone of inhibition (R_F 0.5-0.6) which corresponded exactly with the "reference spot" (revealed by ultra-violet quenching). A similar chromatogram of the combined inhibitory column fractions (2 ml. aliquot), when sprayed with 2,4-dinitrophenylhydrazine reagent, developed a strong orange spot which was the same colour as that given by the violaxanthin oxidation product and with the same R_F value.

The remaining inhibitory fractions were concentrated and acetylated by treatment with acetic anhydride in the presence of pyridine. The chromatographic procedures we have described were repeated using this acetylated product and a mixture of the acetates of (I) and (II). In these tests, inhibition was again shown to occur within the region of the chromatogram which also gave an

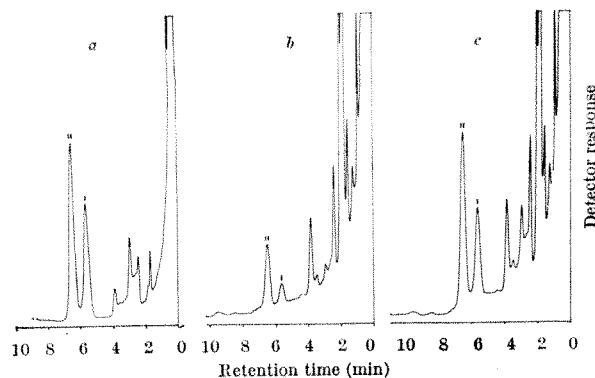


Fig. 2. Gas liquid chromatograms of (a) acetylated violaxanthin photoproducts; (b) acetylated bean tissue extract; (c) a mixture of (a) and (b). Peaks produced by the acetates of the *cis,trans* (I) and *trans,trans* (II) isomers are indicated.

orange spot when sprayed with 2,4-dinitrophenylhydrazine reagent, and at the same R_F as the authentic acetates (about 0.8).

Further evidence for the identity of the dwarf bean inhibitor with the violaxanthin photoproducts was obtained from a comparison of their acetates by gas liquid chromatography. We used a Pye series 104 chromatograph and a 150 cm column of 1.5 per cent XE-60 on 'Gaschrom-Q' at 187° C (50 ml./min flow rate of nitrogen) was used to separate the acetates of the isomers. The acetylated bean extract contained both *cis,trans* and *trans,trans* isomers with the latter predominating (Fig. 2).

The possibility that the bean inhibitor had been formed from xanthophylls during the purification of the plant extracts was investigated separately. Two batches of dwarf bean seedlings (70 g fresh weight each) were extracted with ether. Violaxanthin and neoxanthin, the two xanthophylls which have previously been shown to produce inhibitor on illumination^{3,4}, were also obtained from bean seedlings (350 g fresh weight) and added to one of the extracts. The precise purification procedures we have described were then carried out on the "bean extract" and "bean extract plus pigment". Bioassay tests on these extracts showed conclusively that the five-fold increase in the quantities of violaxanthin and neoxanthin present had not altered the concentration of inhibitor.

Bean seedling extract was also used to investigate the effect of temperature and light intensity during purification. It was shown that an extract processed in a cold room (3° C) with the minimum of red light contained the same quantity of inhibitor as a similar extract purified in normal laboratory conditions. These results indicate that the bean inhibitor is present in the plant tissues and is not formed from pigments during the extraction and isolation processes used.

These extraction, purification and assay methods were used with wheat seedlings which had been grown in the same environmental conditions as those described for dwarf beans. When extracts were chromatographed on silicic acid and the fractions assayed for growth inhibitors, histograms similar to those in Fig. 1 were obtained. Furthermore, examination of the principal inhibitory fractions, as previously described, gave results similar to those obtained with bean seedlings. We therefore conclude that xanthoxin occurs in both normal shoots of dwarf bean and leaves of young wheat seedlings.

Xanthoxin is thus a highly active growth inhibitor occurring naturally in certain plant tissues, and we consider it likely that the compound is formed *in vivo* by the oxidation of xanthophylls. The metabolism of xanthoxin and its possible relationship with (+)-ABA are being investigated in this laboratory.

We thank Professor R. L. Wain for advice and encouragement, and Mr R. P. Townsend and Mr P. Cozens

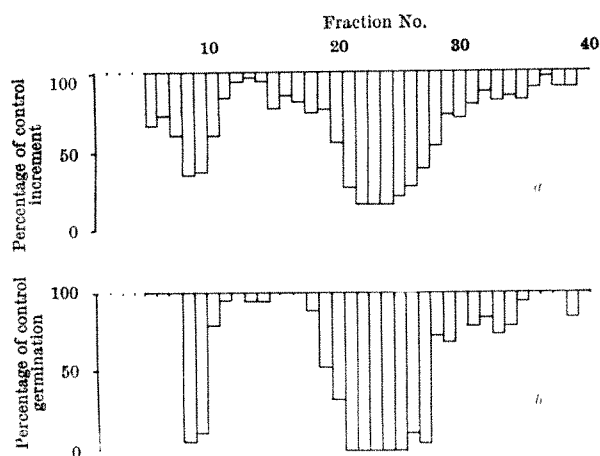


Fig. 1. Inhibitory activity of fractions from a column of silica gel when assessed in (a) the wheat coleoptile section test; (b) cress seed germination test.

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Life Cycle of *Eimeria stiedae*

KNOWLEDGE of the early development of *Eimeria stiedae*, before the appearance of trophozoites in the liver of its rabbit host, is remarkably scanty. After initial penetration of the duodenal epithelium by the sporozoites, the earliest observation of trophozoite formation has been 72 h after infection. The route of migration of the sporozoites during this period has long been a subject for speculation, and the most favoured theory has been passage to the liver from the mesenteric lymph node in the portal blood. Slater, Quisenberry and Fitzgerald¹ gave support to this theory by reporting the presence of sporozoites in the mucosa after 5–9 h, free and in monocytes in the mesenteric lymph nodes and in monocytes in the peripheral blood 16–20 h after infection.

One of the principal technical difficulties in the study of specific coccidia has been the difficulty in keeping experimental animals free of extraneous coccidial infection. The work described in this preliminary communication has been carried out using specific pathogen free (SPF) animals which were entirely free from coccidia, and maintained during experiment in plastic film isolators similar to those used for maintaining gnotobiotic animals. The use of this type of animal and equipment has made possible a different approach to the study of *E. stiedae*.

Previous research was directed largely towards the demonstration of the sporozoites in the tissues by direct staining or labelling. I have tried to trace the spread of the organisms by an indirect method of tissue inoculation from experimentally infected donor rabbits into non-infected recipient rabbits. By maintaining their SPF condition, the recipients were then used as indicators for the presence of viable sporozoites in tissues where they had not previously been demonstrated by other techniques.

Donor rabbits dosed with $2-4 \times 10^6$ sporulated *E. stiedae* oocysts by stomach tube were killed 12, 24 and 48 h after infection, and samples of lymph node and bone marrow were aseptically removed. From the donor rabbits culled after 48 h, liver and blood buffy layer, axillary lymph node and washings from the coelomic cavity were also taken. These tissues were homogenized and injected intravenously into 8 week old recipient rabbits previously sedated with 5 mg of promazine hydrochloride.

Recipients were examined daily for signs of infection, and faecal samples were taken for flotation tests. Animals were killed at the first appearance of oocysts in the faeces or 16–20 days after inoculation if there were no signs of infection.

The results showed that viable sporozoites were present in the mesenteric lymph node after 12 h, in bone marrow after 24 h and in liver and buffy layer after 48 h. Infection could not be induced from the axillary lymph node, washings from the coelomic cavity or from control donors.

Liver tissue taken after 48 h produced severe coccidiosis in test animals. But the apparent presence of sporozoites in blood and bone marrow after 24 h indicates a widespread distribution of organisms with possible involvement of the liver much earlier in infection than previously appreciated. Viable organisms were present in the mesenteric

lymph node as early as 12 h after infection, producing mild hepatic lesions in the test rabbits. The increasing severity of lesions with tissue taken at 24 and 48 h suggests that there may be a steady accumulation of organisms in this tissue.

It is hoped that further tests and quantitative studies on tissue taken at different time intervals after oral infection will help to further clarify the situation regarding the spread of sporozoites during the first 72 h after infection.

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Respiration, Growth and Maintenance in Plants

THE dependence of plant respiration on photosynthesis and dry weight interests the crop scientist, and recently McCree¹, in order to account for his data on white clover, put forward the following simple equation to describe whole plant behaviour

$$R_d = kP_g' + cW \quad (1)$$

where R_d is the dark respiration rate, W is the plant dry weight, and k and c are constants. P_g' is a gross photosynthetic rate which is calculated by using

$$P_g' = P_n + R_d$$

where P_n is the net photosynthetic rate. In this communication equation (1) is derived theoretically using a straightforward extension of Pirt's discussion of yield and maintenance in bacterial cultures². The derivation gives a better understanding of the role of the constants k and c in McCree's equation. The relevance of dark and light respiration to the following analysis (where this distinction is at first ignored) and to McCree's equation is discussed later.

It will be assumed that in a time interval Δt the amount of substrate Δs generated by photosynthesis is $P_g \Delta t$, where P_g is the gross photosynthetic rate, so that

$$\Delta s = P_g \Delta t \quad (2)$$

In a steady state, this increment of substrate Δs must be completely utilized during time Δt . It will be assumed that this substrate has only two uses—maintenance and growth. Thus a part of the substrate (Δs_m) is respired for the provision of maintenance energy, and a part (Δs_g) is used for growth

$$\Delta s = \Delta s_m + \Delta s_g \quad (3)$$

All the substrate appearing in the term Δs_m is respired, whereas only part of the substrate in Δs_g is respired, the rest being turned into plant material. It will be supposed that Δs_g may be partitioned

$$\Delta s_g = \Delta s_r + \Delta s_t \quad (4)$$

where Δs_r is completely respired, and the resulting energy is used to transform Δs_t into plant material (this term includes both structural and storage components) without loss of mass; substrate and plant material are measured in units of CO_2 equivalents. The term, yield (Y), will be used here in the microbiological sense to mean increase in mass in plant material per unit mass of substrate used. The yield of the process of constructive growth (Y_G) is then given by

$$Y_G = \frac{\Delta s_t}{\Delta s_r + \Delta s_t} \quad (5)$$

The observed yield (Y) is (compare ref. 2, equation 1)

$$Y = \frac{\Delta s_t}{\Delta s_m + \Delta s_r + \Delta s_t} \quad (6)$$

From equations (2), (3) and (4)

$$P_g = \frac{\Delta s_m}{\Delta t} + \frac{\Delta s_r}{\Delta t} + \frac{\Delta s_t}{\Delta t} \quad (7)$$

and from equation (5)

$$\frac{\Delta s_t}{\Delta t} = \left(\frac{Y_G}{1 - Y_G} \right) \frac{\Delta s_r}{\Delta t} \quad (8)$$

The maintenance requirement may be expressed in terms of substrate consumed per unit of plant material per unit time. Following Pirt² the maintenance coefficient m is defined by

$$\frac{\Delta s_m}{\Delta t} = mW \quad (9)$$

The respiration rate is given by

$$R = \frac{\Delta s_m}{\Delta t} + \frac{\Delta s_r}{\Delta t} \quad (10)$$

and applying equations (7), (8) and (9) to equation (10)

$$R = (1 - Y_G)P_g + mY_GW \quad (11)$$

which is of the form of McCree's equation.

Another useful form of this equation can be obtained by rewriting it in terms of net photosynthetic rate, P_n . Using

$$P_g = P_n + R \quad (12)$$

one obtains

$$R = \left(\frac{1 - Y_G}{Y_G} \right) P_n + mW \quad (13)$$

The formal relation between the yield constants Y and Y_G is exactly the same in plants as in bacteria (ref. 2, equation 6). Defining the relative growth rate μ by

$$\mu = \frac{1}{W} \frac{\Delta s_t}{\Delta t} \quad (14)$$

then from equations (5) and (6)

$$\frac{1}{Y} = \frac{1}{Y_G} + \frac{m}{\mu}$$

It can easily be shown that the relative growth rate for plants is then given by equations (14), (7), (8) and (9)

$$\mu = Y_G(P_g - mW)/W$$

Rates of respiration in the light are usually greater than in the dark, and it is assumed here that respiration in the light consists of light and dark components, the dark component being unaltered by light. If the light component of respiration is zero then $R = R_d$, $P_g = P_g'$ and equation (11) becomes identical to McCree's equation. If the light respiration is simply due to cycling of CO_2 into and out of the plant with no useful products, then for the present purpose it can be ignored. R_d is used in equations (11), (12) and (13), again giving an identity between McCree's equation and equation (11). If light respiration results from substrate utilization for constructive growth, then this process will probably have a different (lower) yield than the constructive growth component of dark respiration. However, in order to extend the above analysis to cover this case, it is simplest to assume that both components have the same yield, and examine the consequences of this assumption. The respiration rate R (which is really an average respiration rate over time interval Δt) now includes both the light and dark components

$$R = R_d + R_l$$

so that

$$P_g = P_g' + R_l$$

R_l is the light-dependent component of the respiration. If these two relations are put into equation (11), then

$$R_d = (1 - Y_G)P_g' - Y_G R_l + mY_GW \quad (15)$$

This equation can be written in the same form as McCree's equation if it is assumed that the light respiration rate is proportional to the gross photosynthetic rate:

$$R_l = \alpha P_g' \quad (16)$$

which implies that $P_g = (1 + \alpha)P_g'$. Putting equation (16) into (15) gives

$$R_d = [1 - Y_G(1 + \alpha)]P_g' + mY_GW \quad (17)$$

This expression suggests how the role of light respiration might tentatively be assessed from experiment. A direct measurement of light respiration will make it possible to calculate α from equation (16); another value of α can be obtained from a McCree-type of experiment (using equation (17)) if the yield constant Y_G can be measured by some independent means (carbohydrate utilization, for instance); if α from equation (17) is, say, a half of α from equation (16) this indicates that only a half of light respiration results from processes useful to the plant.

McCree¹ found for white clover that $k = 0.25$ and $c = 0.015 \text{ g CO}_2 \text{ day}^{-1}$ (gCO_2 equivalent of dry weight)⁻¹. Applying equation (17), this gives $Y_G(1 + \alpha) = 0.75$. Measured values of yield listed by Warren Wilson (Table 4, ref. 3) are close to 0.75, but are usually a little lower; this possibly indicates (this comparison must be made with caution because of differences in units used) that α is greater than zero and that light respiration contributes significantly to growth. Taking $\alpha = 0$, and using McCree's value for c , the maintenance coefficient m becomes $0.020 \text{ gCO}_2 \text{ day}^{-1}$ (gCO_2 equivalent of dry weight)⁻¹. This compares with a value of about 2 (in the same units) for *Aerobacter aerogenes* growing aerobically on glycerol². This ratio of about 100 between the maintenance coefficients of white clover and *Aerobacter* is reflected by similar differences in growth rate and protein turnover rate between higher plants and microorganisms.

Although this analysis has been applied to the whole plant, the same considerations describe a part of a plant if gross photosynthetic rate is replaced by the rate of supply of substrate.

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Potential Quasi-energy and Energetically Optimal Size in Living Organisms

BIOENERGETICS is concerned with the relationship between the rate of energy exchange by an organism and the other parameters of its body. Perhaps the best known findings are, first, that the total heat production $Q(m)$ increases with increasing body mass according to equation (1) (refs. 1 and 2).

$$Q(m) \approx am^b \quad (1)$$

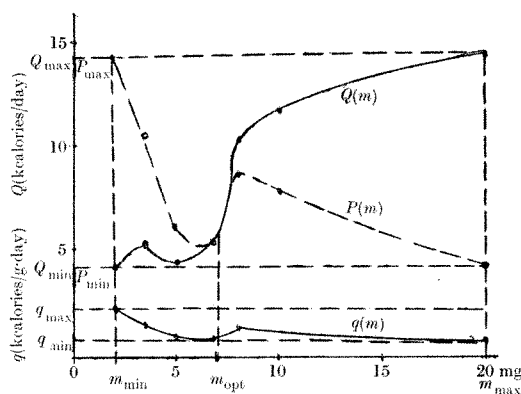


Fig. 1. Total heat production $Q(m)$, specific heat production $q(m)$, and auxiliary function $P(m)$ in shrews.

where m is the body mass, a is the so-called energy level of the organism^{2,3} and b never exceeds 1.00. Second, the specific heat production decreases with increasing body weight according to equation (2) (Fig. 1).

$$q(m) = Q(m) \approx am^{b-1} \quad (2)$$

Several authors have emphasized that in order really to understand evolution it is necessary to know the relationship between the functions² $Q(m)$ and $q(m)$. An attempt at such an analysis is outlined here.

Consider what we shall call the quasi-energy $H(m)$ of an organism of mass m , defined as the product of $Q(m)$ and $q(m)$, that is,

$$H(m) = Q(m) \cdot q(m) \quad (3)$$

When calculated for shrews (Fig. 2) $H(m)$ shows features which are characteristic of potential energy curves, for example, a "potential well" and a "potential barrier", and we feel that these features are of biological significance. Experimental data⁵ suggest that the crucial stages in the life cycle of the shrew, that is survival in winter and the reproductive period in spring, are associated with specific points on the potential quasi-energy curve, the survival period coinciding with the "potential well" and the reproductive period with the "potential barrier".

If one considers a particular value of the body mass, such that a minimal change of mass will move the organism to a "well" or "barrier" on the quasi-energy curve, this may be regarded as the energetically optimal body mass, that is to say, the point of inflexion between m_1 and m_2 may be regarded as an energetically optimal size m_{opt} in the range $[m_{min}, m_{max}]$ for this organism.

Because Fig. 2 is a semi-logarithmic plot, it is clear that optimum size is at approximately the centre of the size range, that is,

$$\log m_{opt} \approx \frac{1}{2}(\log m_{min} + \log m_{max})$$

or, on a linear scale, the optimal size will be the geometric mean, that is,

$$m_{opt} \approx \sqrt{m_{min} \cdot m_{max}} \quad (4)$$

In this case the optimum size is biased towards a low value because the geometric mean is less than the arithmetic. This result also has real biological meaning in so far as the size distribution of individuals in a population and of species in a genus is normal on a semi-logarithmic scale⁶ (Fig. 3) and its maximum coincides with the energetically optimal size.

There are organisms with the same exponent b and the same size range $[m_{min}, m_{max}]$, but with different coefficients a in equation (1), for example, large poikilothermic and homeothermic organisms². The homeothermic animals are generally regarded as having reached the highest

energy level. The concept of quasi-energy makes the definition of the energy level of the organism precise because its curve approaches the reference level H_0 (Fig. 2) and adopts the same values on the boundaries

$$H(m_{min}) \approx H(m_{max}) \approx H_0 \quad (5)$$

Thus we may call H_0 a quasi-energy level of the organism and a step in organic evolution, from the point of view of bioenergetics, would be the achievement of a new quasi-energy level^{2,3}.

Table 1. VARIATION OF MEAN VALUES (M) AND COEFFICIENT (CV) OF THE BODY MASS (g) IN COMMON AND PYGMY SHREWS IN THE KIEV AREA THROUGHOUT LIFE

Months	M	CV	\pm	Scv	No.	M	CV	\pm	Scv	No.
VI	7.3	10.2	\pm	0.90	64					
VII	7.9	10.7	\pm	1.03	54					
VIII	7.4	14.6	\pm	2.43	18					
IX	8.2	12.8	\pm	0.75	144	3.1	10.2	\pm	1.42	26
X	8.5	11.4	\pm	0.45	323	3.2	10.0	\pm	1.03	47
XI	7.1	8.5	\pm	0.59	109	2.7	5.0	\pm	0.87	28
XII	6.8	8.0	\pm	0.48	138	2.6	6.5	\pm	0.76	36
I	6.3	6.3	\pm	0.60	57	2.5	5.2	\pm	1.30	8
II	6.5	10.8	\pm	1.30	35					
III	9.7	13.0	\pm	1.26	53					
IV	12.2	2.3	\pm	0.27	37	4.5	17.4	\pm	3.00	17
V	12.2	5.3	\pm	0.78	23	4.4	10.7	\pm	2.08	8
VI	12.2	11.9	\pm	1.68	25					
VII	11.8	10.9	\pm	2.22	12					
IX	11.8	10.7	\pm	2.38	10					

$CV = 100 \times \sigma/\bar{x}$; $Scv = CV/\sqrt{2n}$.

Other properties of organisms (such as variability and structural energy) are related to the quasi-energy (Table 1 and Fig. 4). Variability proves to be in direct proportion to the quasi-energy level while the structural energy is, on the contrary, in counterbalance with it.

The problem of the relationship between the functions Q and q can alternatively be approached by considering their intersection. Both functions possess the same range of definition $[m_{min}, m_{max}]$, but have different ranges of significance $[Q_{min}, Q_{max}] \neq [q_{min}, q_{max}]$ and dimensions

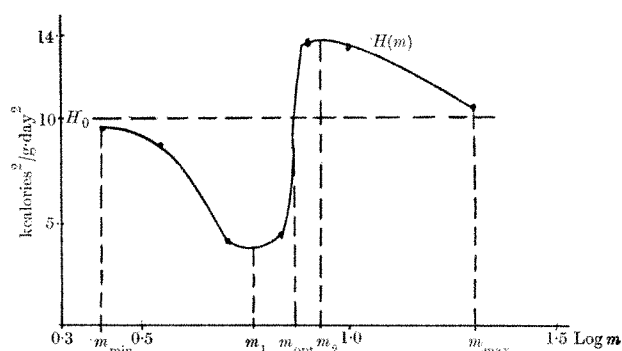


Fig. 2. Potential quasi-energy $H(m)$ in shrews.

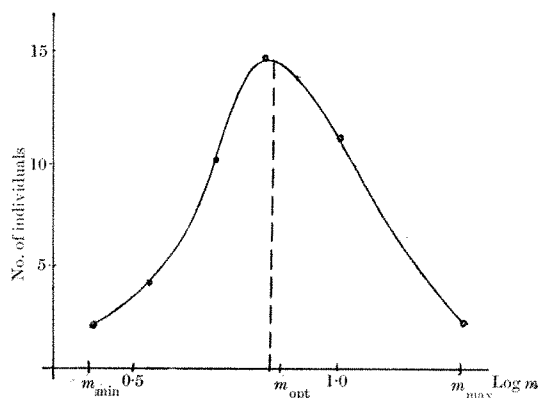


Fig. 3. Dispersion of species in the genus *Sorex*.

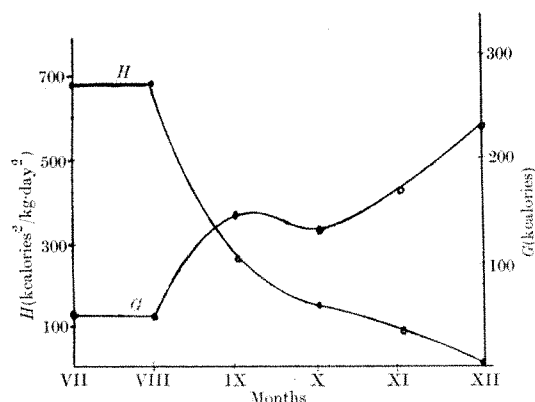


Fig. 4. Structural energy G and quasi-energy H dynamics in *Orchestia bottae* (Amphipoda, Tellioidae)*.

$$[Q] = \frac{\text{kcalories}}{\text{hour}} \neq [q] = \frac{\text{kcalories}}{\text{kg} \cdot \text{h}}$$

If we now introduce a new function $P(m)$, with the same ranges of definition, significance and dimension as the total heat production $Q(m)$, and simultaneously closely related to the specific heat production $q(m)$ according to the linear equation, then

$$P(m) = \alpha q(m) + \beta$$

In this case the linear transformation parameters α and β enable $P(m)$ to meet the conditions of having the same range as $Q(m)$

$$\begin{aligned} P(m_{\min}) &= Q(m_{\max}) \\ P(m_{\max}) &= Q(m_{\min}) \end{aligned}$$

Thus the new function is

$$P(m) = -\frac{\Delta Q}{\Delta q} q(m) + \frac{\Delta H}{\Delta q}$$

where ΔQ , Δq , ΔH represent increments of Q , q , H within the range $[m_{\min}, m_{\max}]$. There is a coincidence point m_0 which can be found from the equation $P(m) = Q(m)$, or

$$-\frac{\Delta Q}{\Delta q} q(m) + \frac{\Delta H}{\Delta q} = Q(m)$$

Using equation (3), this equation can be rewritten in a symmetric form

$$\frac{\Delta Q}{Q(m)} + \frac{\Delta q}{q(m)} = \frac{\Delta H}{H(m)}$$

In our case $\Delta H \approx 0$ (5), so that

$$m_0 \approx -\frac{\Delta Q}{\Delta q} \quad (6)$$

Expressing Q and q in terms of $H(m)$ by means of equations (2) and (3)

$$Q(m) = \sqrt{mH(m)} \text{ and } q(m) = \sqrt{\frac{H(m)}{m}}$$

and using equation (5) anew, we get from equation (6) the point of intersection as

$$m_0 \approx \sqrt{m_{\min} \cdot m_{\max}}$$

which thus coincides with energetically optimal size

$$m_0 \approx m_{\text{opt}}$$

Thus the intersection method gives the same result as a multiplicative one, although at first sight they have nothing in common.

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Measuring the Change of Population Fitness by Natural Selection

Haldane and Van Valen^{1,2} measured the intensity of natural selection by the difference of the mean fitness from the optimum. Haldane used the formula $I_H = \log_e w_0 - \log_e \bar{w}$ where w_0 is the fitness of the optimum phenotype and \bar{w} is the mean fitness. Van Valen's formula is $I_V = (w_0 - \bar{w})/w_0$ which gives values similar to Haldane's. But w_0 may be unknown. The optimum phenotype with the highest fitness may not have occurred in the population. If, for example, among existing phenotypes fitness increased as a linear function of a character, there would be no way of obtaining a value of the optimum phenotype. Values of I_H and I_V would then be arbitrary and depend on an arbitrary value chosen for w_0 .

The intensity of selection is a measure of how far selection has to go to reach whatever optimum—theoretical or actual—happens to be chosen. It is surely of more interest to know what change of fitness occurs during the period within the life-cycle of the organism when the selection is actually taking place. Let w be the fitness of a phenotype and let the character being measured have a phenotypic value of x . Let x occur with probability P . Then by definition the mean fitness is

$$\bar{w} = \sum Pw$$

After selection x has the probability

$$P' = Pw/\bar{w}$$

Therefore the mean fitness after selection is

$$\begin{aligned} \bar{w}' &= \sum P'w = [\sum Pw^2]/\bar{w} \\ &= (V_w + \bar{w}^2)/\bar{w} \end{aligned}$$

where V_w is the variance in fitness. Thus

$$\bar{w}' - \bar{w} = \Delta \bar{w} = V_w/\bar{w}$$

This is, of course, Fisher's fundamental theorem of natural selection³. Because it is applied here only to the selection acting on phenotypes it is exact and general.

I used a model of selection for the optimum of a quantitative character to derive formulae for the changes in the mean and variance of x (ref. 4). The intensity of the selection on x can then be calculated. In this model the fitness is given by

$$w = 1 - \alpha - K(\theta - x)^2$$

where θ is the optimum value of x . When $x = \theta$, w is at its optimum value of $1 - \alpha$. This seems to be the most general model of selection that can be solved for $\Delta \bar{w}$ for any distribution of x . Similar solutions can be derived for other models of w if x is normally distributed. In general, however, x must be assumed to have any frequency distribution with a mean of x and second, third and fourth moments about the mean of V_x , μ_{3x} and μ_{4x} . It is easy to show that if

$$w = 1 - \alpha - K(\theta - x)^2$$

then

$$\begin{aligned} \bar{w}' &= 1 - \alpha - K(\theta - \bar{x})^2 - K V_x \\ V_w &= K^2 \{ \mu_{4x} - V_x^2 + 4 V_x(\theta - \bar{x})^2 - 4 \mu_{3x}(\theta - \bar{x}) \} \end{aligned}$$

and

$$\Delta\bar{w} = V_w/\bar{w}$$

in agreement with Fisher's fundamental theorem.

It can be proved⁴ that

$$\Delta\bar{x} = \frac{2KV_x(\theta - \bar{x}) - K\mu_{4x}}{1 - \alpha - K(\theta - \bar{x})^2 - KV_x}$$

$$(\Delta\bar{x})^2 + \Delta V_x = \frac{KV_x^2 - K\mu_{4x} + 2K\mu_{3x}(\theta - \bar{x})}{1 - \alpha - K(\theta - \bar{x})^2 - KV_x}$$

and from these equations

$$\theta - \bar{x} = \frac{V_x^2(\Delta\bar{x}) - \mu_{4x}(\Delta\bar{x}) + \mu_{3x}[(\Delta\bar{x})^2 + \Delta V_x]}{2V_x[(\Delta\bar{x})^2 + \Delta V_x] - 2\mu_{3x}(\Delta\bar{x})}$$

$$\varphi = \frac{1 - \alpha}{K} = \frac{2V_x(\theta - \bar{x}) - \mu_{3x}}{\Delta\bar{x}} + (\theta - \bar{x})^2 + V_x$$

Normally we only want to know the relative fitnesses of the phenotypes. If we put $\alpha = 0$, the phenotype $x = \theta$ is given a relative fitness of one. Thus $K = 1/\varphi$ and the "fitness function" is

$$w = 1 - (\theta - x)^2/\varphi$$

from which the relative fitness of each phenotype can be calculated. If the absolute mean fitness is known—for example, if the total proportion of survivors during the period of selection is known—then it is a simple matter to calculate α and thus obtain the absolute fitness function.

When the values of $\theta - \bar{x}$ and φ have been calculated, the change in relative fitness can quickly be found. Because $\Delta\bar{w}$ is the change in mean fitness $\Delta\bar{w}/\bar{w}$ is the relative change in mean fitness which is $\Delta\bar{w}/\bar{w} = V_w/\bar{w}^2$. Thus

$$\begin{aligned} \Delta\bar{w}/\bar{w} &= \frac{K^2[\mu_{4x} - V_x^2 + 4V_x(\theta - \bar{x})^2 - 4\mu_{3x}(\theta - \bar{x})]}{[1 - \alpha - K(\theta - \bar{x})^2 - KV_x]^2} \\ &= \frac{\mu_{4x} - V_x^2 + 4V_x(\theta - \bar{x})^2 - 4\mu_{3x}(\theta - \bar{x})}{[\varphi - (\theta - \bar{x})^2 - V_x]^2} \end{aligned}$$

As an example, the change of relative fitness can be calculated for the selection acting on the spotting of *Maniola jurtina*. Dowdeswell's⁵ data of samples of the butterfly before and after selection of the newly emerged adults are given in Table 1.

Table 1. *Maniola jurtina* BEFORE AND AFTER SELECTION

No. of spots	No. of the females	
	Before selection	After selection
0	124	294
1	67	111
2	34	53
3	10	13
4	2	0
Total	237	471

We find $\bar{x} = 0.72996$; $V_x = 0.84202$; $\mu_{3x} = 0.89592$; $\mu_{4x} = 2.65987$; $\Delta\bar{x} = -0.18643$; $\Delta V_x = -0.26573$, giving $\theta - \bar{x} = -2.85546$ and $\varphi = 39.5943$.

By the model, the optimum fitness occurs at the point $x = -2.1255$, an impossible phenotype on the scale of measurement of the character. This is not necessarily unreasonable, however. It is almost certain the selection is acting on some underlying physiological character of which spot number is but a partial manifestation. The optimum of the physiological character presumably corresponds to a spot number of -2.155 which cannot, however, be observed phenotypically in spots. Applying the formula for the change of relative fitness, we get

$$\begin{aligned} \Delta\bar{w}/\bar{w} &= V_w/\bar{w}^2 \\ &= 0.0423 \end{aligned}$$

The fitness has therefore increased by 4.23 per cent by natural selection. If fitness is a linear function of the character, then it is easy to show that $\Delta\bar{w}/\bar{w} = (\Delta\bar{x})^2/V_x$. For the data on *Maniola* this formula gives $\Delta\bar{w}/\bar{w} = 0.0413$, which is in fair agreement with the previous value. This is because the values of x are a long way from their optimum and fitness is increasing more or less linearly.

If x has a normal distribution and the mean is at the optimum so that $\bar{x} = \theta$ and $\mu_{3x} = 0$, then $\Delta\bar{x} = 0$ and

$$\varphi = \frac{V_x(3V_x - V_{x'})}{V_x - V_{x'}}$$

where $V_{x'}$ is the variance after selection. This gives

$$\begin{aligned} \Delta\bar{w}/\bar{w} &= \frac{(V_x - V_{x'})^2}{2V_x^2} \\ &= \frac{1}{2}(1 - V_{x'}/V_x)^2 \end{aligned}$$

For example, Van Valen and Weiss⁶ obtained data showing that between young and old rats the variance in measurements of their teeth was reduced by a proportion $V_{x'}/V_x = 0.188$. Thus fitness increased by 0.0177 or 1.77 per cent. The corresponding formula for the intensity of selection is $I = (V_x - V_{x'})/(3V_x - V_{x'})$ which gives $I = 0.0859$ (ref. 4). Thus fitness would be increased by a proportion $I/(1 - I)$ or 9.40 per cent if all individuals had the optimum size of tooth. But this is an increase in fitness that can never be achieved, for there will always be some variation, environmental and genetic, round the mean. In general, therefore, the increase of relative fitness, $\Delta\bar{w}/\bar{w}$, is a more satisfactory measure, because it gives as a percentage the increase of fitness that has actually occurred.

Table 2. RELATIVE FITNESSES OF PHENOTYPES BEFORE AND AFTER SELECTION

Phenotypic values	Frequencies		Relative fitness	Relative fitness of <i>Maniola</i>
	Before selection	After selection		
x	f	f'	w	w
0	f_0	f'_0	1	1
1	f_1	f'_1	$f'_1 f_0 / f_1 f'_0$	0.69875
2	f_2	f'_2	$f'_2 f_0 f_1 / f_2 f'_0 f'_1$	0.65746
3	f_3	f'_3	$f'_3 f_0 f_1 f_2 / f_3 f'_0 f'_1 f'_2$	0.54830
4	f_4	f'_4	$f'_4 f_0 f_1 f_2 f_3 / f_4 f'_0 f'_1 f'_2 f'_3$	0

Provided there are sufficient numbers in the samples of individuals before and after selection, the relative fitnesses of the phenotypes can be calculated empirically without using any particular model of selection. If the character is a continuous variate it will be necessary to divide its range into suitable class intervals and to calculate the relative fitnesses of the phenotypes in each class interval. They are calculated as in Table 2. These relative fitnesses will, of course, be subject to large sampling errors, for in the sample of *Maniola* the numbers of individuals with three and four spots are very small. The data on *Maniola* give $\bar{w} = 0.8382$ and $V_w = 0.03350$, so that $\Delta\bar{w}/\bar{w} = 0.04768$, which is slightly higher than the value obtained from the model of selection for an optimum. But the values agree within the usual limits of error.

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Book Reviews

SOPHIST'S DELIGHT

Fallacies

By C. L. Hamblin. Pp. 326. (Methuen: London, March 1970.) 55s.

ONE cat has one more tail than no cat. No cat has two tails. Therefore all cats have three tails. This fallacy was put to me by, and indeed explained by, the deputy commander-in-chief at Bomber Command during one of the crises of the Second World War. I now offer it in gratitude to Professor Hamblin; for I find from his book that it belongs to Aristotle's class of "fallacies of equivocation".

Professor Hamblin notes that "There is hardly a subject that dies harder or has changed so little over the years". The weakness of the human mind for fallacy is indeed hardly less eternal than its search after truth; and to ponder such timeless matters is a refreshment for the man of science who knows that most of his work will be superseded within a few years. For in contemplating fallacy he will find himself in company with some of the keenest minds of the past, starting with Aristotle, who discerned thirteen kinds of fallacy, six of them dependent, as is the three-tailed cat, on the misuse of language, and the others on different modes of confusion of thought. Aristotle even described how such confusion may be deliberately effected: to argue at great length so that one's opponent loses the thread, or at such speed that he cannot follow, or to make him angry so that he cannot think clearly. While Hamblin recognizes Stephen Potter as a modern parallel, he might very properly have also mentioned F. M. Cornford, whose *Microcosmographia Academica* vividly bears the Aristotelian stamp on its chapters on argument and on the conduct of business.

Aristotle's list has been augmented through the centuries, especially during the disputations of the schoolmen, who developed much skill in the induction of fallacies by confusion of argument. The "*argumentum ad hominem*" is an example; by its aid, home rule for Ireland could be condemned because its great advocate, Parnell, was an adulterer. And the scope of the exercise is indicated by the further twenty-two "argumenta" on Hamblin's list before he comes to his own "*argumentum ad nauseam*". Once again, to offer a crumb that may have escaped Aristotle (unless with a prescient sense of pun he included it under solecism), I may mention that a scientist in government circles is reputed to have originated an even more devastating device for demolishing his adversary than any in Aristotle's armoury. It was, I think, at a meeting of the deputy chiefs of staff. The scientist had taken the precaution of discovering his adversary's arguments in advance, and he recognized that they were logically superior to his own. His counter was simply to arrive at the meeting early, and to take each deputy chief aside confidentially and tell him what the adversary would say with the implication that this contained a subtle fallacy that would nevertheless, of course, be as obvious to the deputy chiefs as it was to him. The unfortunate adversary therefore presented an excellent case and was bewildered to have it rejected without the scientist having to say a word, simply because each deputy chief had been flattered into pretending that he was clever enough to see the fallacy.

Francis Bacon gave much attention to fallacies in *The*

Advancement of Learning, where he described them as "idols", and Hamblin remarks that Bacon's work was a turning point in their treatment, so that "from now on, some part of the analysis of fallacy will involve an appeal to psychological factors, as in the idols of the tribe and cave, or to social ones, as in the idols of the market place". And, following Bacon's condemnation of the academics who "have tumbled up and down in their own conceits", the Royal Society took as its motto "*Nullius in Verba*". But even the society was not immune to the shrewd attack of Charles II with his famous question about the displacement of water by a live fish in a bowl. This falls into Aristotle's class of "fallacies of many questions" in which the fallacy is presupposed in the question asked; and protagonists of multiple-choice questions would do well to take heed, as Banesh Hoffmann has pointed out in *The Tyranny of Testing*.

Hamblin's account of the Royal Society's floundering over the fish might have benefited from a scientific collaborator who could have resolved for him the question that he poses regarding the weight of a closed cage containing a bird in flight: and this collaboration might have resulted in more discussion of those more interesting fallacies that arise not from any sleight-of-mind, but from the errors that sometimes inevitably arise in the application of scientific method. False correlation is one frequent source, illustrated by Musselwhite's observations that nature had arranged for black men to live in hot climates, and his deduction that Europeans should therefore wear dark suits in the tropics. Such correlations are bound to be made from time to time as science attempts to advance: no scientist, for example, in Western Europe in the 17th century could be blamed for stating that all swans were white. It was a good working conclusion until the discovery of Australia.

The dangers of inductive fallacy are exemplified in the history of vacuum, even beyond the point to which Professor Hamblin takes it. Arnauld in the *Port Royal Logic* (1662) cited the fact that suction pumps could not raise water by more than about 32 feet as evidence against a fallacious conclusion from the ancient observation that nature abhorred a vacuum. But, ironically, Arnauld's own conclusions turn out to be somewhat fallacious, for, even as Hamblin was writing, the National Engineering Laboratory was showing that with due precautions water can be pumped by suction to 56 feet (*New Scientist*, January 29, 1970).

Again, arguments that are qualitatively valid at all scales of physical dimensions may give correct conclusions at one scale but misleading ones when the scale is changed. Brougham supposed, for example, that natives carry burdens on their heads because they had subconsciously discovered that these burdens then weigh less because they are further away from the centre of the Earth (*Nature*, 46, 53; 1892). His argument was qualitatively correct, but the effect is so trivial that it has nothing to do with the action. Again, it may be valid to argue that more light will come out of a gas discharge tube if more gas is present; this is true at low pressures, but not at high ones, where self-reversal may set in. And it may not be a false analogy (a type of fallacy first discussed by Abraham Fraunce in 1588) to suggest that the same may be true of an educational system.

I would have welcomed more attention to Occam's razor. Hamblin does, indeed, mention it; and he tentatively uses it to deal with belief in ghosts, as I myself have used it for flying saucers. But a further discussion of its powers and defects would have been well worth a place. Inevitably, it sometimes leads to error, when it is made to operate on selected evidence, but it is the best tool that we have.

Mathematics is not treated at all in the book; but it would have been interesting to see an analysis of mathematical fallacies, going beyond Canning's dictum that "nothing was so fallacious as facts, except figures". How

many schoolboys, on learning that $e^{2\pi i} = 1$, have boggled at the consequences of taking logarithms?

Hamblin excurses into Indian logic, where fallacies have also had much attention, notably in the *Nyaya Sutra*. This is often printed with a commentary by the author of the *Kama Sutra*, Vatsyayana, who therefore seems to be "from the productions which he left behind him" the oriental equivalent to the younger Gordian (*Nature*, 205, 949; 1965).

This glance at India completes the first half of the book, which is historical and, as the blurb says, is little less than a history of logic itself. In the second half, Hamblin, whose interest in the matter started with Buridan, aims at showing logicians the scope and limitations of logical reasoning; and although the book is not directed primarily to natural science, the man of science may gratefully contemplate its lessons.

R. V. JONES

IMPROBABILITIES

The Implications of Induction

By L. J. Cohen. Pp. vii + 248. (Methuen: London, April 1970.) 75s.

SINCE Francis Bacon, students of inductive logic have generally recognized two types of inductive inference: from numbers and from variety of instances, or induction by simple enumeration and induction by elimination of rival hypotheses. Induction by simple enumeration can be regarded as a special case of inference to population hypotheses from samples, and it has therefore often been assimilated to probabilistic inference, particularly in the confirmation theories of Keynes, Jeffreys, Reichenbach and Carnap. It has, however, been found surprisingly difficult to incorporate into such probabilistic theories the requirement of variety of irrelevant circumstances of observation, which is certainly present in all scientific testing of general hypotheses. Mr Cohen argues that this difficulty is not accidental or surmountable in principle; it is the consequence of a radical distinction between the two traditional methods of induction, which he thinks cannot in principle be assimilated to each other, and which have different logics. His book consists of an argument for, and formal development of, a logic of inductive support, which he claims is appropriate for induction by elimination and is not expressible in terms of any functions of probabilities.

Understood as an explication of eliminative induction many of the *prima facie* puzzling features of this logic become plausible. For example, an immediate and apparently highly counter-intuitive consequence of Cohen's axioms is his so-called "instantial conjunction principle". This states that if any evidence *E* supports a singular substitution instance ("a is a raven only if black") of a universal generalization ("all ravens are black"), then to just the same extent *E* supports the conjunction of any number of such substitution instances ("a and b and . . . are ravens only if black"). That is to say, if observation of a single black raven *a* is support for the assertion of the casual or essential tie which seems to be implied by "a is a raven only if black", then it is equal support for this causal tie in any instance of ravenness. From the point of view of testing of a statistical hypothesis this is absurd, but if the assertion of a cause can somehow be validated by the elimination of all variables other than ravenness which might have been relevant to blackness, it becomes intelligible. To the standard objection to eliminative induction of this kind, that we can never know what the complete list of all such other variables is, Cohen's reply seems to be that its determination is a matter for scientists to judge. What constitutes a "cause" for science is prior to inductive investigation. That this is his view seems to be confirmed by his rather cavalier dismissal of those cruces for a satisfactory theory of

induction: Hempel's and Goodman's paradoxes. Both types of paradox, he argues, depend on misunderstanding the semantics of test-statements for causal laws. But what non-inductive criteria there are for the causal character of laws he does not tell us. This question is so crucial to the inductive theory presented here that absence of any discussion of it makes the theory at best an incomplete contribution to the philosophy of induction.

MARY HESSE

HARTLIB AND HIS CIRCLE

Samuel Hartlib and the Advancement of Learning

Edited by Charles Webster. (Cambridge Texts and Studies in the History of Education.) Pp. x + 220. (Cambridge University Press: London, February 1970.) 40s.

Mathematical and Philosophical Works

By John Wilkins. (Two volumes in one.). (Cass Library of Science Classics, No. 11.) Pp. xiv + 261 + xvi + 265. (Cass: London, February 1970. First published 1708.) 147s.

WHEN Robert Boyle returned from his private education in Geneva to an England in the throes of civil war in 1644, his youthful idealism soon swept him into the orbit of a remarkable Prussian refugee from the Thirty Years' War, Samuel Hartlib. Hartlib was influenced partly by Francis Bacon's vision of a new kind of science that would establish man's dominion over nature; but even more so, by continental reformers like Alstead, Andreae, and Comenius, whose "new science" was at once more mystical and more starkly utilitarian than Bacon's, and was part of a greater social, religious, and educational reform which they believed would be a preparation for the millennium. Boyle's celebrated reference to an "Invisible College" seems to refer to Hartlib and his associates. The calling of the Long Parliament in 1640, and later the victory over the Royalists, signalled for them the imminent realization of the Biblical "New Eden" in England. Hartlib and his group bombarded Parliament with a remarkable range of plans for reorganizing school and university education, agriculture, arts and crafts, and employment and social security.

But with Parliamentary victory came disunity among the reformers, and a dampened enthusiasm for change on the part of the new rulers. Two years before the Restoration, Hartlib wrote to Boyle that he was "wonderous glad that you have written of the present Protector's intentions for contentancing and advancing of universal useful learning in due time"; but he now despaired of realizing his dream, except perhaps in Virginia. Neglected by acquaintances who once found him useful as a "universal intelligencer", and who were now banding together in the new Royal Society, he died in poverty in 1662.

In a long introduction to his selection of Hartlib's papers on the "advancement of learning", Mr Charles Webster has provided an important reassessment of the activities of Hartlib and his associates. His account includes many new details from the unpublished Hartlib Papers, and his reappraisal of Hartlib's influence on the early Royal Society is of particular interest for the history of 17th century English science.

As Webster points out, Boyle always retained something of the religious outlook formed in the Hartlib circle. But he soon moved away from its mystical-chemical and extreme-utilitarian idea of science towards that mechanical and "corpuscular philosophy" which he was to be so influential in propagating. Boyle's taking up lodgings in Oxford about 1651 clearly marked the break. He came at the invitation of John Wilkins, "intruded" Warden of Wadham College, who asked him to join the youthful, scientific group which flourished at Oxford since the late 1640s.

Wilkins showed little appreciation of the revolutionary conceptual innovations of Galileo and Descartes which were the inspiration of the most gifted members of his group. Except for a distaste for mystical chemistry, his ideal of science resembled that of Hartlib's. He looked to it primarily for mechanical devices and inventions to advance trades and crafts. Nevertheless, he was an important representative of the gentleman-amateur tradition of 17th century English science, and the new and reasonably priced republication of his works illustrates his role in popularizing and defending the Copernican system, and the astronomical discoveries of Tycho, Kepler, and Galileo. In 1638, he foresaw the time when man would make a passage to "the World in the Moon":

"Yea, but (you will say) . . . we have not now any Drake, or Columbus, to undertake this voyage, or any Daedalus to invent a conveyance through the air.

"I answer, though we have not, yet why may not succeeding times raise up some spirits as eminent for new attempts, and strange inventions, as any that were before them?"

P. M. RATTANSI

SPACE PHYSICS

Cosmic Ray Physics

Nuclear and Astrophysical Aspects. By Satio Hayakawa. (Interscience Monographs and Texts in Physics and Astronomy, Vol. 22.) Pp. xi + 774. (Wiley (Interscience): New York and London, February 1970.) 370s.

THE subject matter of the series of biennial conferences on cosmic ray physics organized by the Cosmic Ray Commission of IUPAP has in the past shown very clearly the changing interests in the field over the years. Before 1953, fundamental particles and high energy collisions were the dominant topics for discussion whereas, from 1953 onwards, the emphasis has been very much on the geophysical, heliophysical and astrophysical aspects of cosmic rays. This change has been partly the result of the appearance on the scene of high energy laboratory accelerators and partly because of the advent of satellites and space probes which permit cosmic ray observations outside the magnetosphere and have led to an enormous increase in our understanding of the field and particle environment in space.

About a half of Professor Hayakawa's book is devoted to the physics of high energy collisions, including fundamental particles and electromagnetic interactions, and in this part there is collected together a great deal of useful and readily accessible information in the form of graphs and formulae. The subject of high energy interactions is still in the developing phase and the author presents a convenient account of both the relevant experimental data and of current theories. This is followed by chapters on the cosmic ray beam in the atmosphere and underground and on large cosmic ray air showers. Finally, there is a long section, almost 200 pages, dealing with the astrophysical aspects of cosmic rays and the problems of the cosmic acceleration process and the origin of the particles.

It is hardly surprising that in a book of this length there are some obscurities, errors and inaccuracies. For example, on p. 11, there is no clear distinction between history and current understanding, with the result that the reader is left with the impression that Forbush decreases are caused by the ring current—a suggestion made in 1937 and discarded as incorrect quite soon afterwards; on p. 159 elementary particles are categorized as belonging to the familiar four groups of photon, lepton, meson and baryon, but unfortunately in the accompanying table (pp. 160–161), which lists the particles, the term baryon nowhere appears and pions appear under the heading leptons; on p. 532, the trapping of energetic

particles in the solar system is discussed in a semi-quantitative way, but without any reference to the convective effects of the solar wind which, in fact, dominates the situation. There is often a marked absence of discussion of the physical ideas underlying the mathematical formulae, which number well over a thousand, and some parts of the book, which was completed in the autumn of 1966, read very much like lecture notes. Indeed, the author points out in the preface that some sections are based on courses of lectures he has given. It is by no means the most readable book that has been produced on cosmic ray physics and is certainly to be recommended for use as a reference book rather than as light reading. H. ELLIOT

BORN'S VIEW OF PHYSICS

Physics in My Generation

By Max Born. (Heidelberg Science Library.) Pp. vii + 172. (Longman: London and New York, April 1970.) 50s.

"THE idea of collecting these essays occurred to me when, in the leisure of retirement, I scanned some of my own books and found that two of the more widely read showed a startling change of attitude to some of the fundamental concepts of science", writes the author in the preface. The essays are mostly reprints of articles or records of lectures given on various occasions; they may have been addressed to laymen in science or to Nobel Laureates.

As a frame to the book, Born has chosen the "Introduction to Einstein's Theory of Relativity" (1921) and the "Postscript to the Restless Universe" (a young person's book on elementary atomic and nuclear physics dedicated to his son Gustaf, 1951), from the two books referred to earlier.

The first, the principal part of the book, gives much insight into the development of physical concepts in the fifty years from 1901 onwards. Born has himself been an active participant in formulating quantum mechanics and, though he did not himself take part in working in general relativity, his friendship with Einstein was originally based on Einstein feeling "completely understood and appreciated", and this led to a lifelong discussion on basic issues. The dramatic evolution in scientific thought comes out well in these essays; they are therefore enjoyable and valuable not only for scientists but also for those interested in the history of the materialistic world picture. Most of the essays in this section have previously been published under the same title by the Pergamon Press in 1956.

The second part contains four essays of a different slant, Born's concern with the problems of nuclear energy. A man of great sensitivity and wide interests, he was plunged into deep depression and despair over the possibility that the applications of modern science might lead to the extermination of mankind. Thus not only the laws in physics had changed from deterministic ones to statistical ones, but the proud attainments of the natural sciences had become of doubtful value.

EGON BRETSCHER

HAMILTON'S THEORY

An Introduction to Hamiltonian Optics

By H. A. Buchdahl. (Cambridge Monographs on Physics.) Pp. xv + 360. (Cambridge University Press: London, March 1970.) 120s; \$18.50.

THIS book contains the best account of Hamilton's theory that I have come across. Applications to electron optics as well as to ordinary optics are considered, and the author keeps to a pleasant lecturing style of writing, although the nature of the subject matter (which is often heavily algebraical) makes this difficult at times.

There are twelve chapters, each with a problems section

at the end. Outline solutions to the problems are given at the end of the book. Printing and diagrams are excellent.

Chapter one, a short one, deals in an introductory way with Fermat's principle. The second chapter introduces Hamilton's point characteristic V and the general notion of a characteristic function, then the angle characteristic T , the "mixed" characteristics W_1 and W_2 , and finally the notion of an aberration function. Chapter three discusses the relation between symmetries of optical systems and invariance properties of characteristic functions. Ordinary centred systems, of course, provide the most important practical examples, but the discussion is more general. Chapters four and five develop the theory of the symmetric system (the ordinary "centred system") and chapter six that of systems with additional symmetries. Chapter seven discusses semi-symmetric systems, which have an axis of symmetry but no plane of symmetry containing the axis. Here the medium is necessarily anisotropic. (Electron microscopes using magnetic focusing provide an example of this type of system.) Systems with other types of symmetry are discussed more briefly in the following two chapters. In chapter ten, a fairly short discussion of chromatic defects of the image is given. Chapter eleven deals with the effects of anisotropy, such as can occur in crystalline media.

The last chapter, on the computation of aberration coefficients, begins with an interesting discussion on whether the Hamiltonian or the Lagrangian method of computation should be considered the best practical basis for optical (and dynamical) computations. So far as optical computations are concerned, the author is probably better qualified than most experts to pass judgment on this question. He says: "The great simplicity of the Lagrangian method leads me to believe that it, rather than the Hamiltonian method, is best adapted to the problem of practical calculations; though this is admittedly a mere expression of opinion". Nevertheless, because of the interest of this question and also to round off the treatment, he goes on to give one possible procedure of calculating characteristic functions, that is to say the coefficients of their power series.

The book is an outstanding contribution to the "classical" literature of the subject and deserves a place in every optics library.

E. H. LINFOOT

ABELIAN GROUPS

Infinite Abelian Groups

By Laszlo Fuchs. Vol. 1. (Pure and Applied Mathematics, Vol. 36.) Pp. xi + 290. (Academic Press: New York and London, January 1970.) 140s.

THE rate at which group theory is growing is indicated by the fact that the author, intending to prepare a revised edition of his 1958 book on Abelian groups, found that in order to take reasonable account of work done during the past decade a completely new book must be written. His new volume should therefore be particularly useful to the young postgraduate student, for in many places it brings him up to the frontiers of present-day knowledge, shows him modern methods and, in addition, offers a number of explicit research problems to challenge him. The reader should have some knowledge of abstract algebra, set theory, and topology; from this point onwards the book is self-contained, but a previous acquaintance with the fundamental properties of Abelian groups and with the type of austere abstract reasoning involved, while not absolutely essential, is highly desirable. The author has striven for clarity of expression, and has not thought the provision of numerous exercises for the reader beneath the dignity of a volume intended for postgraduate study; these are genuine exercises, not

sections of theory for which no room could be found in the main text.

The influence of homological algebra is evident; the 1956 book on this topic by Cartan and Eilenberg unified cohomology theory for groups and classes of algebras and helped to clarify and simplify certain aspects of group theory. The concepts of categories and functors supply generality, and the diagrammatic representation of homomorphisms gives an intuitive grasp of complex relationships.

The first volume deals chiefly with important classes of Abelian groups, direct sums, divisible groups, and the like, and extension problems and torsion products. The second volume is to deal with structure theory and applications.

T. A. A. BROADBENT

HYDRATION PROCESSES

Hydration and Intermolecular Interaction

Infrared Investigation with Polyelectrolyte Membranes. By Georg Zundel. Pp. xi + 310. (Academic Press: New York and London, January 1970.) 168s.

THIS volume summarizes the results of investigations by infrared spectroscopy carried out at the University of Munich on the molecular processes involved in hydration, particularly of polyelectrolytes in the form of cation-exchange resins.

The assignment of the absorption bands of polystyrene, polystyrenylsulphonyl chloride and of polystyrene-sulphonic, -selenic, -phosphinic and -thiophosphonic acids and their salts is fundamental to the interpretation of the measurements, and is therefore analysed critically. A careful examination is also made of the effect of hydrogen-bonding on the spectra of water and deuterated water, as well as of such phenomena as the effects of the location of the cation with respect to the oxygen atoms of the anion and of the degree of hydration on the stretching vibrational frequencies of polyelectrolyte anions. The effects of hydration and of association of acid groups on the spectra of the acids are collected and discussed. It is concluded that the two bands always occurring, which must be ascribed to OH or OD groups in the hydrogen-bridges connecting the acid groups, cannot be caused by a double minimum in the potential energy curve, but arise from a stretching vibration and an overtone of the bending vibration of the OH and OD groups. The natures of the various forms of hydrated proton which occur in these systems and the tunnelling effects in proton migration are discussed. Finally, the book gives details of the apparatus and techniques used in preparing cation-exchange membranes by production of a polystyrene membrane of definite thickness and degree of cross-linking and subsequent introduction of the desired acidic groups. The techniques used in the investigation of such membranes by infrared spectroscopy are also discussed.

The book provides an extremely important summary of the subject for those interested in the structures of these systems, but its price will seem high to British readers.

J. W. SMITH

CHEMICAL MARINE BIOLOGY

The Chemical Biology of Fishes

With a Key to the Chemical Literature. By R. Malcolm Love. Pp. xv + 547. (Academic Press: London and New York, January 1970.) 140s; \$21.

CHANGES in the external environment such as those of salinity, oxygen tension, temperature and pressure pose special problems to the maintenance of a state of homeostasis (constancy of internal environment) by fishes over the whole life cycle. This book provides a much needed survey and discussion on the chemical composition of tissues and body fluids of wild fish, as well as of fish that

have been at least partially acclimatized to the more standard conditions of life in an aquarium.

Chapter one is entitled "Towards a Valid Sampling Technique" and illustrates the problems encountered by research workers in obtaining animals in a sufficiently unstressed condition, from the sea or an aquarium, to determine what might be regarded as a normal range of concentration. This difficulty is apparent in almost all of the subsequent chapters when the author goes on to discuss the quantitative and qualitative changes in the chemical composition that occur during the life cycle, the effect of changes in the external environment and the effects of voluntary or involuntary starvation. Next, some differences between species are discussed and finally there are two appendices. The first appendix occupies 4 pages and consists of a simple, useful but somewhat uncritical index to references where individual chemical compounds have been detected in a wide variety of fishes. The second, of 16 pages, details the familial relationships of fish genera and gives the common and the systematic name as well as some qualitative indication of the chemical compounds that have been identified in the animal.

The aim of the book has been to relate a chemical and an analytical approach to the general biology of fishes. The selection of material for discussion has obviously been influenced to some extent by the author's own extensive research work and his own personal viewpoint. This is all to the good because the text is clearly and often entertainingly written in a highly individual style, but it should be noted that the features of metabolism that distinguish fish from other animals and the nature of fish enzymes have not been considered in detail.

To sum up, this monograph is an extensive reference work on the chemical composition of fishes up to 1968. It will be a valuable source of information to research workers and students who have interests in marine biology.

P. T. GRANT

THE ANIMAL WORLD

Animal Senses

By Robert Burton. Pp. 183+18 plates. (David and Charles: Newton Abbot, March 1970.) 65s.

THERE has always been a fascination in considering what the world must look, smell and sound like to animals. Is their world similar to ours, or is it a caricature of the detailed sensations which we feel? With the pace of electrophysiological investigations on sense organ function and the quantity of information coming from experiments on perception, there is almost a continuing need for reviews of this subject couched in terms which the layman understands. The author of this book has undoubtedly set out with this aim in mind, but in execution has come sadly wide of the mark. There are too many misleading statements and inaccuracies for the book to be recommended. One way in which the author errs is in the misapplication of terms which have quite specific meanings; thus, the explanation of kinesis on p. 19 is quite misleading, the performance of a worm in a T-maze is not a demonstration of a conditioned reflex, and a catalyst does not "supply energy".

In line with modern ideas the author describes experiments on sense organs but too often leaves out important details; for example, one electrode is insufficient to detect impulses in a nerve; where is the other one? With the anatomy and function of sense organs, mistakes again appear. Thus the hearing organs of grasshoppers (if we take the popular English usage of the word) are not in the legs, and the vibrations striking the antenna of a male mosquito are not transmitted down it but the whole flagellum is set into motion and it is the tuning of this flagellum which makes it so responsive to the wing note of the female. The illustrations are often over-simplified sketches of the true complexity of the sense organs;

glaring examples are the figures of the hearing organ of the grasshopper (which also shows the receptors in the wrong position) and of the facial pit of a viper.

One last strange feature of the book is the selection of entries in the bibliography; most of these are original papers which must be difficult for most of the potential readers to obtain. There are a number of short books which have been published within the past three years which would have been far more helpful for anybody looking for more information.

J. D. CATHY

DEFINING ECOLOGICAL CONCEPTS

Diversity and Stability in Ecological Systems

Report of Symposium held on May 26-28, 1969. (Brookhaven Symposia in Biology, No. 22.) Pp. vii+264. (Clearinghouse for Federal Scientific and Technical Information, NBS, US Department of Commerce: Springfield, Virginia.) \$3.0.

ONE of the pressing problems facing theoretical ecologists is the construction of a precisely defined framework on which to hang their data. Ecology is still dogged by vague, quasi-philosophical terms which lend themselves to a multitude of individual interpretations; two of such terms are diversity and stability. These concepts, together with their relationship to one another, form the theme of a symposium held at Brookhaven, New York, in May 1969. This volume is a valuable collection of nineteen papers from that symposium, given by a number of leading ecologists, and it serves to emphasize the need for unified models of ecological systems which will allow precise, mathematical formulation of our current embryonic concepts of ecosystem function.

Most of the authors interpret diversity in terms of the MacArthur model and take stability to mean the ability of a system to withstand and absorb major environmental perturbations. Contention arises in the definition of a major perturbation, and Lewontin helps to elucidate this by constructing a theoretical model in which there are several possible "basins of attraction" into which a system can settle; thus when perturbation becomes sufficiently violent the system may move to a new basin and achieve a new equilibrium. Margalef goes further and proposes a function which expresses stability in terms of biomass and attrition of that biomass.

Other papers, for example, those by Miller, Sanders and Slobodkin, are concerned with the origins of diversity, and all conclude that environmental stress leads to lowered diversity, whereas predictable environments (for example, the sea bed) allow a highly diverse, biologically accommodated biota to develop. Wilson provides experimental demonstration of this principle in his study of arthropod invasion of defaunated mangrove islands.

The time factor in the definition of stability is a familiar problem and Goulden shows long term increases in diversity by a study of fossil Cladocera in lake sediments, a subject further elaborated by Deevey.

A number of field situations are analysed in detail, for example, Watt's work on the behaviour of populations of insects and of epidemic diseases. He demonstrates an interesting, if enigmatic, correlation between upsets in population stability and volcanic eruptions. Cantlon concentrates on the effects of technology on natural populations of organisms, which provides a link with several papers dealing with the importance of stability and its accompanying (causative?) diversity to the survival of man on this planet. Some profitable thoughts emerge from these papers, though attempts to elevate ecology to a political system ("biocracy"—Caldwell) or even a religion ("not peace—but ecology"—Hardin) must be viewed with caution.

The volume is sensibly priced, poorly arranged and well worth reading.

PETER D. MOORE

PLANT CUTICLES

The Cuticles of Plants

By J. T. Martin and B. E. Juniper. Pp. vii + 347. (Arnold: London, March 1970.) 150s.

UNTIL recently there has been a relatively small literature available on the anatomy, chemistry and function of plant cuticles. By contrast there is a voluminous literature dealing with the holes that occur in cuticles because studies of the factors that affect the opening and closing of plant stomates have long been a favourite topic for investigation. With the development of the technique of gas chromatography, the availability of commercial electron microscopes, and the discovery of the procedures of freeze-etching and carbon replica formation, it is now possible to make intensive studies of the waxy cuticles of plants. This is currently an active research area and the authors of this book are to be congratulated on preparing a good book at the right time.

The combination of a chemist and a botanist has resulted in a wide cover of the literature because they have listed more than 1,000 references that impinge on their subject. For some, possibly too much emphasis has been placed on experimental methods, especially in the early chapters, but for others these chapters will be an invaluable source of references. There are few significant omissions; possibly the physics behind cuticular resistance to water vapour loss could have been better developed, and information on the actual magnitude of the resistance values could have been given in view of the great importance of the plant cuticle in controlling desiccation. Mention might also have been made of "chemical pruning agents" used in floriculture to kill selectively terminal meristems because these compounds almost certainly exert their effect due to the differences that exist between the cuticles of young and old tissues. A surprising inclusion is the interesting but relatively inaccessible work on the waxy cuticles of *Eucalyptus* sp. taken from an unpublished PhD thesis by Hallam.

I particularly like the introductory paragraph at the start of each chapter to help orientate the reader for what is to come. The cross-references within the text, the table of binomial and vernacular names and the glossary are also helpful. There is no tendency for the authors to simply tabulate a sequence of research findings as, throughout, current thinking has been summarized and presented as well-digested arguments.

I believe that the stated objectives of the authors in writing a book "of interest to all plant biologists and especially to workers in plant physiology, plant pathology, crop protection and to university, college and school teachers" although a rather large task has, in the main, been achieved. Finally, the publishers are to be commended for the high technical quality of the book; in particular, for the eye-catching dust cover, for the use of glossy paper throughout and, especially, for the high quality of the reproduction of electron micrographs, as carbon replicas of wax surfaces notoriously lack contrast. There is every chance that this book will become and will remain the standard reference on plant cuticles for quite some years to come and, as such, represents good value for money.

J. V. POSSINGHAM

HANGERS-ON

The Biology of Parasitic Flowering Plants

By Job Kuijt. Pp. 246. (University of California Press: Berkeley, Los Angeles and London, November 1969.) 143s.

PARASITIC flowering plants occupy a significant place in the flora of the world and in the agricultural economy of many tropical and sub-tropical regions, yet they form the one group that has been sadly neglected from the point of view of a unified presentation of the accumulated knowledge.

This book succeeds in filling this gap by surveying the whole field of parasitism by higher plants, from the lowly hemiparasite to the almost science fictional existence of *Rafflesia* and its relatives. Every aspect of the biology of these plants receives some attention.

An introductory chapter gives an absorbing account of the discovery of parasitism and the folklore, uses and medical and magical properties of parasitic plants. The author then allocates a chapter to each of the principal groups of parasitic flowering plants and makes a detailed examination of their important families and genera. The groups considered are the mistletoes; the sandalwoods; the broomrapes and figworts; the Rafflesiaceae, Hydnoraceae and Balanophoraceae—the families with relatively large parasitic complements; and *Cuscuta*, *Cassytha*, Lennoeaceae and Krameriaceae—isolated parasitic genera and families with few parasitic species. For each family the patterns of and variations in embryology, seed structure and germination, flower structure, fruit structure and dispersal, and the habit and mode of parasitism are described. These descriptions are illustrated by many excellent line drawings, mostly drawn by the author from material in the herbarium of the University of California, Berkeley. The half-tone illustrations, however, lack clarity on the matt paper, and many of them should merit special treatment in a book costing as much as this one.

Subsequent chapters deal with the structure of the haustorium, and the physiological and evolutionary aspects of parasitism. The foremost of these chapters describes the development and structure of the absorptive organs of the parasites, by which nutritional contact with the host is made. The author puts forward, in this chapter, a comparative account of haustorial structure. Absorptive organs are described, from the simple haustorium of the unspecialized root parasites, through the intricate haustoria of the specialized holoparasites, to the mycelium-like endophytic systems of the Rafflesiaceae. He concludes that, although the parasites display a marked degree of evolutionary plasticity in this respect, the haustorium of parasitic angiosperms represents a root in function and evolutionary origin. The physiological aspects of parasitism considered are germination and the exudation of germination stimulants by the roots of potential hosts, the nutrition and water economy of parasites, host ranges and the effects of parasites on their hosts. The final chapter discusses the possible evolutionary pathways of parasites and the relations between the structure of parasites and their evolutionary position.

The noticeable predominance of structure over function in this book is a reflexion not only of the author's research interests but also of our lack of knowledge in the fields of the physiological and nutritional aspects of parasitism and host relations.

The bibliography of some 700 references and the very detailed index combine to complete a book which is very readable and a long awaited reference book in this fascinating aspect of botany.

R. N. GOVIER

FOULING OF AN ESTUARY

Effects of Abatement of Domestic Sewage Pollution on the Benthos, Volumes of Zooplankton, and the Fouling Organisms of Biscayne Bay, Florida

By J. K. McNulty. (Studies in Tropical Oceanography, No. 9.) Pp. 107. (University of Miami Press: Florida, 1970.) n.p.

A STUDY of the ecological effects of pollution has to be comparative; unfortunately, by the time it becomes clear that such a study is needed, it is usually too late to observe the unpolluted condition. Dr McNulty has taken an unusual opportunity to make such a study in reverse.

The population of Miami increased from less than 2,000 to nearly 250,000 between 1900 and 1950, when 30 to 50 million gallons per day of its domestic sewage were being discharged into the Miami River or directly into the northern part of Biscayne Bay, which contains the port of Miami and separates it from Miami Beach. At the river mouth, coliform bacteria exceeded 100,000 per 100 ml. The impact of this pollution on the tourist industry eventually stimulated public demand for a treatment works. When plans were announced in the early 1950s, a series of surveys were made; the works began operating in 1956 and the surveys were repeated in 1960-61.

Benthic communities showed the greatest changes. At the periphery of a zone of organic pollution, such as existed in the mouth of the river, the food supply is abundant. Oxygen, which might otherwise have been limiting, was supplied by strong tidal currents, but most predators were excluded by conditions which were nevertheless abnormal. Dense populations of tolerant amphipods, polychaetes and lamellibranchs therefore developed. When the pollution was abated, diversity returned and the abundance of these tolerant forms was rapidly reduced. A marked reduction was also observed in the red algae typical of this community, but evidence from adjacent areas suggests that extensive dredging and perhaps hurricane damage may account for this. In the shallow and poorly flushed parts of the estuary, the concentration of inorganic phosphate and plankton volume both declined considerably, but elsewhere plankton showed little change between the two surveys. Changes in fouling organisms were generally unrelated to pollution, although there was a reduction in the numbers of tube-building amphipods. The unusual concentration of potential bottom food was not utilized by fish of commercial or sporting value, so the discharge had no fertilizing effect on the fisheries.

The volume is bound in thick boards and is well documented with tables and diagrams but, excluding these, there are only some 26 pages of actual text. The title pages, contents and prefatory material are well spaced and are not excluded from the pagination, so that the introduction (reviewing much of the previous work on estuarine pollution and eutrophication problems) begins on p. 19; a bibliography of 155 references extends over 15 pages. It might thus be considered an expensive item for a general library, but will prove useful to the specialist, particularly as little has yet been published about the effects of pollution in tropical waters.

A. NELSON-SMITH

ADVANCES IN POLLUTION CONTROL

Advances in Environmental Sciences

Edited by J. N. Pitts jun. and R. L. Metcalf. Vol. 1. Pp. 356. (Wiley (Interscience): New York and London, March 1970.) 150s.

THE recent explosion of interest in pollution has produced many new popular and scientific journals, as well as a host of books. In most branches of science we have "Annual Reviews" or "Recent Advances", and the volume considered here indicates that pollution will also have its multi-volume series. There is no indication, however, of the frequency with which new volumes will appear. If this means that publication will only take place when enough new material is available, the series will be welcomed by scientists if not by librarians, who may find it difficult to budget for an irregularly appearing and rather expensive periodical.

The editors are well chosen for their task. Professor Pitts is an authority on air pollution, with particular experience of photochemical smog. Professor Metcalf is an entomologist, with an international reputation in insect control. Unfortunately, they do not seem to have decided exactly for whom their series is intended. They themselves have contributed, as an introduction to their first

volume, an "Outline of Environmental Sciences" which is a useful summary which might have appeared, with little modification, in one of the British quality Sunday newspapers.

The other papers are of an uneven standard, and will appeal to readers with very different backgrounds. Thus the paper on the role of the American federal authorities in pollution control, by John Tunney, a member of congress, is purely administrative, though it sketches in the background against which scientists will have to work. Professor Kruse of Johns Hopkins University gives an account of the problems relating to American water supplies. This contains information which might be difficult to extract quickly from the literature, but there is little information which has not been described before, though the account could be valuable to non-scientists.

On the other hand, other articles assume that the reader has considerable chemical knowledge. Dr Edgar R. Stephens deals in detail with the peroxyacyl nitrates (PANs) in photochemical smog, and one of the longest papers, by Dr James N. Pitts, considers the importance of singlet molecular oxygen as an environmental oxidant, perhaps explaining some of the anomalies considered by Stephens. This latter paper contains much that will be incomprehensible to any except a specialist in the author's own field.

Not all the articles are particularly relevant to present-day conditions. Thus Dr Theodore E. Brenner of the Soap and Detergent Association devotes 50 pages to an account of biodegradable detergents. None of the data included is less than four years old. Most countries solved the "detergent swan" problem some years ago, and the facts are already included in standard textbooks. It is disappointing to note that there is no mention here of the current worry about eutrophication, particularly by phosphates, from modern, biodegradable, detergents.

Future volumes in this series may serve a useful purpose. Volume one suggests that the title "Advances in Environmental Sciences" is well chosen, instead of the alternative "Recent Advances" used in some comparable publications.

K. MELLANBY

PESTICIDE JOURNAL

Pesticide Science

Vol. 1, Nos. 1 and 2. Two-monthly. (Society of Chemical Industry: London, 1970.) Members 80s; non-members 160s per volume. (Per issue, 27s 6d.)

WITH sources of scientific information proliferating rapidly, the principal justification for the appearance of any new journal is that it will enable work to appear under one cover which hitherto has been distributed widely but thinly in numerous other journals. The more interdisciplinary a subject, the greater the tendency for this fragmentation to occur. The problems so created are well illustrated by those of workers in the highly topical and important subject of pesticides, for a balanced approach to this broad-spectrum discipline requires the cooperation of chemists, biochemists, soil scientists, mathematicians and biologists, as well as the special skills of industrialists familiar with practical and financial aspects of the manufacture, field application and commercial formulation of pesticidal substances.

Pesticide Science is published by the Society of Chemical Industry, a society which, by the establishment several years ago of a Pesticides Group, has already done much to encourage an integrated approach to the study of pesticides. The society is also well known for the *Journal of the Science of Food and Agriculture* (which often contains articles on crop protection chemicals) and for the pesticide sections of the *Reports on the Progress of Applied Chemistry*. Its experience is reflected in the quality of the editorial board of the new journal—D. Woodcock, J. K. Eaton,

C. G. L. Furnidge and A. J. Low are four eminent members of the board, selected for mention here because they represent distinct yet overlapping interests in the field of pesticides. The new journal considers for publication work relating to the production and use of pesticides, including those used in veterinary and public health. It also accepts articles covering the ecological and economic implications of their use. Papers in the first two issues of volume one do indeed cover a wide range of interests. It is, however, noteworthy that, while the effect of pesticides on organisms receives attention in several excellent articles, these first two numbers do not seem to do full justice to the complementary subject of the effect of organisms on pesticides. Yet such practically important matters as field performance, persistence, safety and selectivity of action are frequently dependent on the rate and the route a substance is metabolized. The editorial board of *Pesticide Science* undoubtedly recognizes this fact, and it is to be hoped that this apparent weakness in these first two issues is a consequence of pure chance, rather than to the absence of the word "metabolism" from the list of subjects which authors are told will be considered for publication.

With this one reservation, *Pesticide Science* promises to be a valuable new medium both for disseminating and for acquiring information on this rapidly expanding multidisciplinary subject.

KENNETH HASSALL

FIGHT THE GOOD FIGHT

The Ecologist

Edited by E. R. D. Goldsmith. Vol. 1, No. 1. Monthly. (Ecosystems: London, July 1970.) 4s per issue; 40s per annum.

THE bandwagon of environmental crusaders has had the brakes on lately, in Britain at least, but the publication this month of the first issue of *The Ecologist* promises a further lunge forward into new seas of effluent and deserts of dereliction. The magazine is by no means the detached and cautious scientific review that its title might suggest; indeed, insofar as it deals with ecology at all, *The Ecologist* dwells in a man-centred ecosphere and largely in those parts of it which man is supposed to be damaging beyond repair. What the editor, Mr Edward Goldsmith, is anxious to communicate is "the fact that there is a serious emergency", to be attacked fundamentally by checking the growth of population and by dispelling the myth of unrealistically high standards of living.

To this end, Mr Goldsmith has set up his own publishing company, Ecosystems Ltd, which is producing 60,000 copies of the first issue of *The Ecologist* for sale on the bookstalls or by subscription. In the contributed articles, environmental hypochondria is kept well under control. Professor R. Lindsay Robb, for example, argues the case for closer links between medicine and agriculture, suggesting that since both practices are concerned with human welfare they should be controlled by a common ministry. Mr Robert Allen, the assistant editor, also writes illuminatingly about the conflicting interests of the Eskimos and the oil companies in Alaska.

Some of the editorial comment, however, is on less firm ground. "Organisms that obtain mastery over their environment become over-specialized"—from which it is paradoxically made to follow that "they are at the mercy of the slightest environmental change that might create a situation to which they no longer have the means of adapting". Another item deplores the amount of money invested in art, in comparison with the difficulty of raising cash for conservation, and talks of "pseudo-concepts" of which "one of the most pernicious . . . is anthropocentric evaluation". This aggressive creed, coming from a magazine which is called *The Ecologist* but whose subject matter is principally man himself, may seem just a little surprising.

ROBERT MAYCOCK

Short Notices

The Origins and Growth of Modern Education. By Elizabeth Lawrence. Pp. 393. (Penguin: Harmondsworth, April 1970.) 10s.

MODERN educational ideas are not modern at all—they owe their heritage to the Romans, and their line can be traced unbroken through the Roman, Renaissance and Victorian eras. Elizabeth Lawrence, who has investigated the pedigree of "progressive" educational ideas with all the thoroughness of a genealogist, supports her theory with so many quotations that they take up more space than basic text. Twenty-five centuries of educational thinking are packed into the book, and educationalists from Socrates to A. S. Neill of Summerhill school play their part in the lineage. Although entrenched opponents of progressive education are unlikely to be won over by appeals to history, this book provides a useful compendium of educational quotations.

Scienza e Tecnica. Annuario della EST, Enciclopedia Scienza e della Tecnica. Pp. 566. (Mondadori: Milan, 1970.) 14,000 lire.

THE third in this series of scientific yearbooks maintains the outstanding qualities of its predecessors, notably in the eminence of its contributors and the excellence of the design and illustrations. The section on space, for example, contains articles by Dr Donald Rea of NASA on the exploration of Mars and by Dr Vladimir Kurt of the University of Moscow on that of Venus; there are also articles on the exploration of Jupiter and the outer planets, the launch system for the Apollo missions and the guidance and propulsion of space vehicles. The other sections of the yearbook, all of them of equal or nearly equal merit, are devoted to astrophysics, geophysics, the environment and philosophy of science. Even at 14,000 lire (£9 7s) the book is of good value.

The Wild Mammals of Malaya and Offshore Islands, including Singapore. By Lord Medway. Pp. xix + 127 + 15 plates. (Oxford University Press: London and Kuala Lumpur, June 1970.) 125s.

IN the states of Malaya and the adjacent offshore islands, including Singapore, it is illegal to capture or kill the Javan and Sumatran rhinoceroses, the apir, the pangolin, the binturong, three species of gibbon and the slow loris, and there are also several other species of wild land mammals out of a total of 199 in the region that are protected in some way. It would be wise therefore for the naturalist intent on a game hunt to read this book, which should give him sufficient clues to an animal's identity—a brief description is accompanied by notes on distribution, habits and life history, and there are handsome illustrations by Mazli Matsom and Hamidah Suhaimi.

The Royal Botanic Garden Edinburgh, 1670–1970. By H. R. Fletcher and W. H. Brown. Pp. xvi + 309 + 20 plates. (HMSO: Edinburgh and London, 1970.) 72s.

STUDENTS of the history of science whose appetite for botanical memorabilia was whetted by the recent article in *Nature* on Edinburgh's Royal Botanic Garden (226, 904; 1970) will find the full story of three centuries of floristic endeavour in this scholarly book. This is the story of the development of a forty foot square physic garden, founded in 1670 by Andrew Balfour and Robert Sibbald, into seventy-five acres of plants, greenhouses, exhibition areas and research facilities. It is also part of the story of botany's disengagement from medicine and its emergence as a science in its own right, as each new keeper and curator brought new plants and ideas to Edinburgh. The text is enhanced by a fine collection of plans and pictures which illustrate many phases of the garden's growth.

Correspondence

The Fly in the Fourier

SIR,—Long before the publication of my heresy, or *odium theologicum* as it has been termed (*Nature*, **226**, 404; 1970), I realized that there would be reaction from the Establishment; however, I did not expect that this would sink to the level of an anonymous report, cited above, written, apparently, by one unversed in the pitfalls of Fourier analysis. The crystallographic “nuances” referred to are not such at all, but truisms well known to experienced structure analysts. The author of this report appears to be so stunned by the fact that someone could have the temerity to question evidence which has been presented in support of a DNA structure that he has taken leave of his objectivity. Your readers should refer to the original papers (*Science*, **165**, 1091; 1969; **167**, 1693, 1694, 1694, 1700; 1970) and not rely on your reporter’s eclectic treatment of them. Thus, he states, referring to electron density difference maps: “These make the best of the data”, a statement which could be made only by one unfamiliar with the details of this technique. He repeats the fiction that there was misrepresentation of earlier results by omission of reference to difference maps, when, in fact, all parties agree that these contain no more information than do observed and calculated maps, which were found in 1991, above. He characterizes the letter by Crick (above, 1694) as an “olympian boot”, but is so untutored in the nature of diffraction that he is unaware of an egregious misconception of basics in that letter (pointed out in 1700, above) which effectively rendered that boot imperceptible. He also appears to agree with Crick’s view that if I am dissatisfied with the existing “canonical” DNA model I should employ my energy in attempts to construct satisfactory alternative models. But this is not the issue: it is rather like expecting someone who reacts unfavourably to an inadequate production of *Die Götterdämmerung* to mount his own ideal production. The real point, which your reporter so cunningly evaded, is whether the various electron density functions which have been presented as proof of a particular DNA structure do, in fact, constitute proof of that structure. The answer to this question surely should be made by “the trade” with its “crystallographic dialectic”, and not by workers in an unrelated discipline.

Your reporter’s view appears to be that because “the innocent bystanders” in “the outside world of molecular biology” do not understand the recondite world of X-ray crystallography, it would seem best for them to ignore the results of experiment, and believe only what they wish to believe on emotional grounds. This is indeed true innocence.

Yours faithfully,

JERRY DONOHUE

Department of Chemistry,
University of Pennsylvania,
Philadelphia, Pennsylvania 19104, USA.

This letter has been shown to the author of the article concerned, who replies as follows:

Dr Johnson on a celebrated occasion allowed a hot potato to fall from his mouth onto his plate with the words, “Many a lesser man, sir, would have swallowed that”. Evidently this view does not commend itself to Professor Donohue, for he has swallowed his hot potato. His letter, like his reply to his critics (*Science*, **165**, 1700; 1970), reiterates, but does not add to his arguments, and does not answer the charge of biased presentation of data—the failure to mention the part of an alternative structure

which does not fit the data, the use of electron density contour intervals so wide as to vitiate any inferences, and so forth. Nonetheless, Professor Donohue has shifted his ground. After thundering from the rooftops to an awestruck multitude the dreadful (if uncontested) truth that the structure of DNA has not been rigorously proven, he now—a trifle disingenuously, as it seems to me—belabours me for my indelicacy in intruding into a private rift within the crystallographic family, which should not be exposed to the vulgar gaze of the scientific *canaille*. While adopting a posture of defiance in the face of the Establishment, he utters a plea to be judged only by “the trade” or, as it is more aptly termed, the crystallographic Establishment. For the benefit of those readers who flagged before reaching the peroration of Donohue’s article (*ibid.*, 1700), I quote: “I did not mean to imply that the X-ray data for DNA could be fitted just as well by a model with alternative base-pairing. What I said was ‘The Fourier method of structure refinement has in fact contributed nothing toward either the proof of that structure nor toward the elucidation of its details’.” A modest claim, perhaps, and of a truly theological character.

The essential issue, however, was whether Donohue’s strictures can be said to impinge on molecular biology at large, and my argument—though as Donohue rightly implies readers can take it or leave it—was that they do not, because of the bulk and variety of the evidence on base-pairing in both DNA and RNA, and now indeed because of his failure, despite, one must suppose, fairly strenuous efforts, to produce an alternative pairing scheme that fits the data.

I am resigned to the eternal shame of being pilloried, in company with Crick, for my inadequate grasp of basic principles of X-ray diffraction. As to the difference Fourier maps, however, and what is agreed by “all parties”, I would merely direct readers’ attention to the view expressed by four of the parties (*ibid.*, 1693). Though as for *Götterdämmerung*—Professor Donohue, I can only surmise, sees himself as Siegfried, but who, I wonder, will be his Hagen?

Bombs and Earthquakes

SIR,—On May 9, 1970, Dr D. S. Robertson wrote to the *New York Times* pointing out that two severe earthquakes had followed underground nuclear explosions. Since then there has been much uninformed speculation in the press as to whether the Peruvian earthquake could have been triggered by the French nuclear test at Mururoa Atoll on the previous day (May 24, 1970). Robertson¹ is reported to have stated that “it now seemed almost certain that the tests and quakes are correlated”. It is the purpose of this note to produce both general and statistical arguments which lead to the opposite view.

The general reasons depend on the following arguments: the energy put into the ground by the Mururoa Atoll test (a few kilotons in the atmosphere) would be very small compared with that generated by an earthquake of magnitude 4 of which there are an average of 10,000 a year. Tests in the atmosphere are poorly coupled to the ground and we estimate the French test on May 29 to be equivalent to a local event of magnitude not greater than 2.5 of which there are approximately 100,000 every year. If Robertson’s conjecture were correct it would seem that earthquakes would be far more likely to be triggered by other large distant earthquakes than by nuclear bombs, but we know of no evidence or suggestions that this occurs. Small local foreshocks and aftershocks which

often precede and follow larger earthquakes might be considered as examples of these sorts of phenomena, although we believe they are more likely to be part of the total mechanism producing the original earthquake, and in any case they are local, not distant. There is also the fact that, as we would expect, the French atmospheric nuclear tests on May 15, 22 and 29 were not detected by the Warramunga seismic array or to our knowledge by any of the other seismic stations in Australia. Our estimates lead us to believe that the ground movement it would cause in Peru would be far less than the random background movement (seismic noise) which is always present and certainly less than a magnitude 4 event in, say, California. Fairly large earthquakes are not uncommon in Peru; for example, a magnitude 7.4 event occurred on November 10, 1946, and 5.9 on July 24 and October 1, 1969. On any mechanism the time delay, of the order of a day, between the bomb and the explosion required by Robertson would be difficult to explain. Finally, we know that large underground nuclear explosions produce small local shocks² so we should not expect small poorly coupled atmospheric explosions to produce large distant earthquakes.

Statistical evidence has already been cited by Underwood, Cleary and Read³. They found that "the occurrence of large earthquakes in the few days following an explosion is no greater than the normal level of activity". As further evidence we wish to add the following statistical argument:

Assume that earthquakes and bombs are independently distributed. Then the expected number of "coincidences", λ , due to chance is given by

$$\lambda = (\text{No. of earthquakes}) \times (\text{proportion of bomb days})$$

There are forty earthquakes a year whose magnitude is 6 or greater. Since January 1964 there have been 350 nuclear explosions; that is, the proportion of bomb days is approximately 1 in 8;

$$\text{therefore } \lambda = 40 \times \frac{1}{8} = 5$$

On the average, we would thus expect five coincidences a year due to chance alone. The actual number of coincidences a year will be approximately a Poisson distribution with the following probabilities:

Number of coincidences a year	3	4	5	6	7
Probability	0.13	0.16	0.17	0.15	0.12

From this we see that 3, 4, 5, 6 or 7 coincidences a year are nearly equiprobable and that the probability of 3 to 7 coincidences a year is 0.73. This means that we could expect 3 to 7 coincidences to arise by chance alone about every 1.3 years and this is what Robertson seems to have observed.

Yours faithfully,

GORDON NEWSTEAD
MARGARET PILE

Research School of Physical Sciences,
Australian National University,
Canberra, ACT, Australia.

¹ Robertson, D. S., *Canberra Times* (June 8, 1970).

² *Science*, **166**, 601 (1969).

³ Underwood, R., Cleary, J., and Read, L., *Canberra Times* (June 10, 1970).

Announcements

University News

Dr J. G. Thompson, at present senior research fellow at Churchill College, **University of Cambridge**, has been elected Rouse Ball professor of mathematics, as from January 1, 1971.

R. Buckminster Fuller, a member of the faculty of the University of Southern Illinois, will join the **University of Detroit** for one year as visiting professor.

Professor Robert W. Steel, professor of geography at the **University of Liverpool**, has been appointed a pro-vice-chancellor of that university.

Dr D. V. Bugg, principal scientific officer at the Rutherford Laboratory, has been appointed to the chair of nuclear physics at Queen Mary College, **University of London**.

Professor S. J. G. Semple, professor of medicine at St Thomas's Hospital Medical School, has been appointed to the chair of medicine tenable at the Middlesex Hospital Medical School, and **Dr K. O. Kemp**, reader in structural engineering at University College London, has been appointed to the Chadwick chair of civil engineering tenable at that college. The following titles have been conferred: professor of cytochemistry, on **Dr S. J. Holt** in respect of his post at the Middlesex Hospital Medical School; professor of clinical anaesthesia, on **Dr M. K. Sykes** in respect of his post at the Royal Postgraduate Medical School; professor of planning studies, on **Dr P. D. Cowan** in respect of his post at University College London; professor of endocrine physiology and pharmacology, on **Dr J. R. Hodges** in respect of his post at the Royal Free Hospital School of Medicine; professor of crystallography, on **Dr J. W. Jeffery** in respect of his post at Birkbeck College; professor of theoretical computation, on **Mr P. J. Landin** in respect of his post at Queen Mary

College; professor of mathematics, on **Dr G. L. Watson** in respect of his post at University College.

Dr Clive Rosendorff, research fellow of the American Heart Foundation and the British Heart Foundation and visiting assistant professor at Yale University during 1969-70, has been appointed to the Otto Beit chair of physiology at the **University of the Witwatersrand**.

Appointments

Mr J. P. Graham, deputy director of research of the **British Coke Research Association**, has been appointed director in succession to **Dr G. W. Lee**, who retired on June 30.

Mr A. F. Kelly has been appointed deputy director of the **National Institute of Agricultural Botany** in succession to **Dr P. S. Wellington**, who becomes director on October 1.

Dr John W. Fitchie, deputy director of the Fighting Vehicles Research and Development Establishment, has been appointed director of the Transport Research Assessment Group at the Ministry of Transport's **Road Research Laboratory**, in succession to **Dr A. J. H. Hitchcock**.

Miscellaneous

Dr Irmgard Flugge-Lotz, Stanford University, has received the 1970 achievement award of the **Society of Women Engineers**, for her significant contributions to the field of fluid mechanics, in particular wing theory and boundary layer theory.

The president of the **Argonne Universities Association** has announced the establishment of AUA distinguished

appointment programme for service at the Argonne National Laboratory. Each year an award will be available to a qualified faculty member to spend a year at Argonne National Laboratory. A similar award will be available for a qualified Argonne staff member to spend a year at an AUA university of his choice. Selections will be based on outstanding scientific or engineering accomplishments. In addition to his normal support, each recipient will receive a merit award of \$5,000. Nominees must be from the fields of biology, chemistry, engineering, mathematics or physics.

Entries are invited from any British firm or individual for a national competition, sponsored by the **Royal Society of Arts** in conjunction with the **BETRO Trust** and the **Edgar E. Lawley Foundation**, which aims to show the value of innovation in helping Britain's export trade and balance of payments. Two prizes of £500 and two of £250 will be awarded for the best case studies, including the successful use of new designs, inventions or discoveries, which either have contributed significantly in this regard or will do so in the next few years. Further details can be obtained from the Royal Society of Arts, John Adam Street, Adelphi, London WC2N 6EZ.

At the twenty-sixth annual general meeting of the **Institution of Metallurgists**, **Professor A. G. Quarrell**, professor of metallurgy in the University of Sheffield, was elected president of the Institution, in succession to **Dr A. H. Sully**. Also elected were **Dr W. E. Duckworth**, director of research, Fulmer Research Institute, and **Dr H. W. Kirkby**, director of research, Firth Brown Limited (vice-presidents); **Dr E. G. West** (honorary treasurer); **R. J. Brown**, British Leyland (Austin-Morris) Limited, **A. McConnell**, Weir Engineering Industries Limited, and **Professor J. C. Wright**, University of Aston in Birmingham (ordinary members of council elected by the Fellows); **Dr E. R. Petty**, Sheffield Polytechnic, and **E. H. Watson**, British Steel Corporation (ordinary members of council elected by the Associates).

At the annual general meeting of the **Australian Academy of Science**, **Professor R. N. Robertson**, master of University House, Australian National University, Canberra, was elected president of the Academy in succession to **Professor Dorothy Hill**. Other members of the council were also elected: **Professor A. J. Birch** (treasurer); **Professor G. M. Badger** (secretary, physical sciences); **Professor A. K. McIntyre** (secretary, biological sciences); **Dr A. L. G. Rees** (secretary, international relations); **Professor S. T. Butler**, **Dr S. D. Hamann**, **Professor B. Y. Mills**, **Professor B. H. Neumann** and **Professor A. E. Ringwood** (ordinary members, physical sciences); **Professor W. H. Elliott**, **Professor Dorothy Hill**, **Professor G. J. V. Nossal**, **Professor W. P. Rogers** and **Professor R. O. Slatyer** (ordinary members, biological sciences). Six new Fellows of the Academy were also elected, in recognition of their outstanding published contributions to research in the natural sciences: **Professor E. J. Hannan**, Australian National University; **Dr Mollie E. Holman**, Monash University; **Professor D. O. Jordan**, University of Adelaide; **Dr J. F. A. P. Miller**, Walter and Eliza Hall Institute of Medical Research, Melbourne; **Dr W. C. Swinbank**, CSIRO Division of Meteorological Physics, Melbourne; **Dr G. K. White**, CSIRO Division of Physics, Sydney.

ERRATUM. In the article "Advantages of an Antagonist" (*Nature*, 226, 1199; 1970) two references were incorrectly cited. The correct citation for Obata *et al.* is *Exp. Brain Res.*, 4, 43 (1967) and that attributed to Werman *et al.* (*Life Sci.*, 4 2075; 1965) was actually due to Aprison and Werman.

International Meetings

July 21, Memorial Meeting in Memory of the late Professor Sydney Chapman, London (Press Officer, Imperial College of Science and Technology, College Block, London SW7).

July 27–August 7, Contemporary Optics (summer course), Rochester, NY (Contemporary Optics, Institute of Optics, University of Rochester, Rochester, New York 14627, USA).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Furnished 4–5 bedroom house preferably near Orpington, Kent, or in South London area, for Canadian physicist and family (6 children), from September 1, 1970, until April 1, 1971. Please contact Professor L. Krause, 5 rue La Bruyère, 78 Versailles, France (telephone 951-5100), or c/o Dr A. E. Kiss, 14 Elm Grove, Orpington, Kent (telephone 66-20329).

Wanted: Furnished London house with four or more bedrooms for one year beginning September, by returning professor and family. Please contact E. Cumberbatch, Mathematics Department, Purdue University, Lafayette, Indiana, USA; after mid-August, Mathematics Department, University College, Gower Street, London WC1.

Wanted: Furnished 3-bedroom apartment in Paris, accessible to Latin Quarter, from October 1, 1970, to June 1, 1971. Please contact Professor J. J. Price, Mathematics Department, Purdue University, Lafayette, Indiana, USA; after mid-August, 53, Promenade des Anglais, Nice, France.

Wanted urgently: Furnished accommodation for Russian doctor, wife and 1 year old child in London with easy access to King's College, London. Prefer garden flat with central heating. Please contact Dr V. Lednev, Biophysics Department, King's College London, 26–29 Drury Lane, London WC2 (telephone 01 836 8851).

Wanted: Furnished house near western edge of London or suitably situated for feasible commute to National Physical Laboratory, Teddington, and/or University of Reading, from about September 1970 for approximately nine months for visiting fellow, wife, and two children (8 and 10). **Vacant or exchange:** Furnished house in Berkeley, 3 bedrooms, study, 2 bathrooms, well equipped kitchen, central heating, excellent view of San Francisco Bay, for same period plus summer 1971. Please contact Dr P. Concus, Lawrence Radiation Laboratory, University of California, Berkeley, California 94720, USA.

Vacant: 4 bedrooomed house in Surrey, 45 minutes from central London. Free accommodation in return for running the house (owner remaining). Would suit junior postgraduate with not more than two young children. Available now for 12 months. Please contact Dr D. H. Clarke, 18 Starrock Road, Coulsdon, Surrey, CR3-3EH.

British Diary

Monday, July 20

Dielectric Materials, Measurements and Applications (five-day conference) Institution of Electrical Engineers, in association with the Institute of Physics and the Physical Society, and the Institute of Electrical and Electronics Engineers, at the University of Lancaster.

The Mechanisms of Reactions in Solution (five-day international conference) Chemical Society, at the University of Kent at Canterbury.

Tuesday, July 21

Memorial Meeting in tribute to the memory of the late Professor Sydney Chapman, FRS (3 p.m.) in the Department of Physics, Imperial College, Prince Consort Road, London SW7.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

- The Society of Comte: An Appreciation. By E. E. Evans-Pritchard. Pp. 26. (Manchester: Manchester University Press, 1970.) 7s 3d. [86]
- Department of Education and Science. Index to Department of Education and Science Circulars and Administrative Memoranda current on 1st January, 1970. (List 10.) Pp. 51. (London: HMSO, 1970.) 7s 6d (37.5p) net. [86]
- Agricultural Research Council. Annual Report of the Meat Research Institute, 1968/1969. Pp. 73. (Langford, Bristol: Meat Research Institute, 1970.) [96]
- University of Oxford. Report of the Committee on the Long-Term Problem of Entitlement. Pp. 28. (Supplement No. 8 to the *University Gazette*, Vol. C, June 1970.) (Oxford: The University, 1970.) 5s. [96]
- Building Research Station Digest, No. 117: Vibrations in Buildings—1. Pp. 8. (London: HMSO, 1970.) 9d. [96]
- Cotton Research Corporation. Progress Reports from Experiment Stations, Season 1967/68. Malawi. Pp. 20. 2s 6d. Progress Reports from Experiment Stations, Season 1967/68. Republic of the Sudan. Pp. 46. 2s 6d. Tanzania—Eastern Cotton Growing Area. Pp. 22. 2s 6d. (London: Cotton Research Corporation, 1969 and 1970.) [106]
- Family Planning Association. 38th Report and Accounts, 1969/1970. Pp. 48. (London: Family Planning Association, 1970.) 2s 6d. [106]
- BBC Wildlife Series No. 4: Highland Birds. Selected and Introduced by Eric Simms. (Gramophone record.) (London: BBC Radio Enterprises, 1970.) 21s 6d. [116]
- The Zoological Record*, 1968, Vol. 105, Section 15: Pisces. Compiled by G. Palmer and the Staff of the Zoological Society of London. Pp. vi+275. (London: The Zoological Society of London, 1970.) 60s. [126]
- The Mathematical Principles Underlying Newton's *Principia Mathematica*. By D. T. Whiteside. (Being the Ninth Gibson Lecture in the History of Mathematics delivered within the University of Glasgow on 21st October 1969.) Pp. 28. (Glasgow: The University, 1970.) 10s net. [126]
- British Museum (Natural History). Instructions for Collectors No. 2A: Birds. By C. J. O. Harrison and G. S. Cowles. Illustrated by A. L. Dahl. (Publication No. 561.) Pp. 48. (London: British Museum (Natural History), 1970.) 4s (20p). [126]
- Department of Agriculture and Fisheries for Scotland. Index of Collectors in the Edinburgh Herbarium. Edited by I. C. Hedge and J. M. Lamond. Pp. v+147. (Edinburgh and London: HMSO, 1970.) 62s net. [126]
- BBC Lunch-time Lectures Eighth Series—6: The BBC and Its Public. By Kenneth Lamb. Pp. 14. (London: BBC, 1970.) [156]
- Building Research Station. Current Paper 12/70: High-magnesia Portland Cements—Studies on Cements Prepared with Reagent-grade Chemicals. By S. S. Rehs and A. J. Majumdar. (Reprinted from *The Magazine of Concrete Research*, 1969, Vol. 21, June.) Pp. 67–78. (Garston, Watford: Building Research Station, 1970.) [156]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 266, No. 1176 (11 June 1970): Metabelian Groups and Varieties. By R. A. Bryce. Pp. 281–355. 40s; \$5.20. Vol. 266, No. 1177 (11 June 1970): The Nuclear Explosive Yields at Hiroshima and Nagasaki. By Lord Penney, D. E. J. Samuels and G. C. Georgie. Pp. 357–424+plates 2–9. 48s; \$6.25. (London: The Royal Society, 1970.) [156]
- Proceedings of the Royal Irish Academy. Vol. 69, Section B, No. 7: A Swarm of Caledonian Dolerite Intrusions in the Tallaght Hills, Co. Dublin. By N. S. Angus and J. C. Brindley. Pp. 165–178+plates 9 and 10. 4s. Vol. 69, Section B, Number 8: Anticancer Agents—V-Stereoisomeric Methylglyoxal Hydrazone Derivatives. By Joan Byrne and J. F. O'Sullivan. Pp. 179–184. 2s. Vol. 69, Section B, No. 9: Anticancer Agents—VI—Further Hydrazone Derivatives of Methylglyoxal. By Vincent C. Barry, M. L. Conalty and J. F. O'Sullivan. Pp. 185–196. 3s. (Dublin: Royal Irish Academy, 1970.) 2s. [156]

Other Countries

- Smithsonian Contributions to Paleobiology, No. 4: Distribution of Planktonic Foraminifera in the Vicinity of the North Atlantic Current. By Richard Cifelli and Roberta K. Smith. Pp. iii+52. \$0.65. Smithsonian Contributions to Zoology, No. 28: Some Behaviour Patterns of Platyrhine Monkeys. 2: *Saguinus geoffroyi* and some other Tamarins. By M. Moynihan. Pp. iv+77. \$1.25. No. 34: Sublittoral Gammaridea (Amphipoda) of the Hawaiian Islands. By Laurens Barnard. Pp. vi+286. \$3. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) [46]
- US Department of the Interior: Geological Survey. Professional Paper 613-F: Permian Sphenopsids from Antarctica. By J. F. Rigby. Pp. iii+12+3 plates. \$0.45. Professional Paper 657-A: Structural and Stratigraphic Significance of the Buchia Zones in the Colyear Springs-Paskenta Area, California. By David L. Jones, Edgar H. Ballet and Ralph W. Inlay. Pp. iii+24+5 plates. \$0.55. Professional Paper 655-C: Error Analysis of Streamflow Data for an Alluvial Stream. By D. E. Burkham and D. R. Dawdy. Pp. iii+13. \$0.35. Professional Paper 662-A: The Hydraulics of Overland Flow in Hillslopes. By William W. Emmett. Pp. iv+68. \$0.75. (Washington, DC: Government Printing Office, 1969 and 1970.) [46]
- Parliament of New South Wales. Report of the Department of Agriculture for the year ended 30 June, 1969. Pp. 176. (Sydney: Government Printer, 1969.) [46]
- US Department of the Interior: Geological Survey. Professional Paper 613-D: Ferns From the Chinle Formation (Upper Triassic) in the Fort Wingate Area, New Mexico. By Sidney R. Ash. Pp. iv+52+5 plates. \$1. Professional Paper 680-A: Deep Drilling on Midway Atoll. By Harry S. Ladd, Joshua I. Tracey, Jr., and M. Grant Gross. Pp. iii+22+1 plate. \$1. (Washington, DC: Government Printing Office, 1969 and 1970.) [56]
- World Health Organization. Technical Report Series, No. 446: Clinical Pharmacology—Scope, Organization, Training—Report of a WHO Study Group. Pp. 21. 2 Sw. francs; 4s; \$0.60. No. 447: WHO Expert Committee on Plague—Fourth Report. Pp. 25. 2 Sw. francs; 4s; \$0.60. (Geneva: WHO, London: HMSO, 1970.) [86]
- Studia Forestalia Suecica, Nr. 80: A Genealogical Investigation of the Annual Rhythm of *Pinus sylvestris* L. By Mats Hagner. Pp. 40. 8 kr. No. 81: A Genealogical Investigation of the Annual Rhythm of *Pinus contorta* Dougl. and a Comparison with *Pinus sylvestris* L. By Mats Hagner. Pp. 26. 6 kr. No. 82: The Intra-Provenance Correlation Between Annual Rhythm and Growth of Single Trees of *Pinus sylvestris* L. By Mats Hagner. Pp. 40. 8 kr. (Stockholm: Skogshögskolan, Royal College of Forestry, 1970.) [86]
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What Future for the Social Sciences?

It is not an entirely indecent question to ask whether the social sciences will ever become respectable in the narrow sense in which the word is usually understood by academics. One difficulty, perhaps unavoidable, is that the objective of a great deal of social science must be to clarify matters of urgent importance to society. Inevitably, a great deal of research in the social sciences is therefore bound up with the necessary muddy problems of the real world. Whatever changes there may be in the pattern of the work of the Social Science Research Council in the years ahead, there is no danger that it will ever have to grapple, as the Science Research Council has had to do, with the task of making grant recipients more aware of the practical implications of what they do. In this sense, of course, respectability is nothing to be proud of. A more serious worry, not merely for the Social Science Research Council but for the rest of the academic world, is that the social sciences seem often to drift rudderless across the academic scene. Too much of what is done in the universities in the name of the social sciences is neither relevant to the great social issues of the modern world nor helpful in the development of a body of coherent understanding.

Why are the social sciences such a mess? One obvious difficulty is the sheer diversity of the problems to which solutions seem accessible. The grants awarded by the Social Science Research Council in the past year are a splendid illustration of this. At the University of Nottingham, for example, new grants deal with statistical forecasting techniques in economics and the methods of work of the Prices and Incomes Board as well as with studies of child development and urban practices of child rearing which are closer to the universities' traditional interests. Elsewhere are studies of women's fashions, "the microecology of selected hamlets and villages in eastern Botswana" and also a study of "the role of the headmaster". It would be wrong for outsiders to pretend to know that any of these projects does not deserve support—indeed, one of the most impressive features of the latest annual report from the Social Science Research Council (see page 648) is the way in which the council has faced head-on the difficulties which arise from the diversity of its parish and from the inevitable unpreparedness of its pensioners. Without saying much, the council quite rightly behaves as if its first task is to build up a social science community in Britain which is strong enough not merely to hold its head above water but to do so with some pride.

In circumstances like these, however, it is entirely proper to question the council's decision not to pursue a policy of selective support, at least for the time being. Two separate issues arise. First, should sup-

port for research in the social sciences be distributed in such a way that there is a chance that busy policymakers will be helped in their work? This is the argument of those who hold that the social sciences are potentially even more potent than technology as a means of making a better society. The trouble is that the argument puts the cart before the horse in assuming that the magic wand of money will make good ideas appear wherever it is waved. The council is right to reject this criterion for support. The second, more practical issue is whether the council should select a few areas on which to concentrate its efforts, either because these are fields in which good people are already hard at work or because there is reason to believe that further knowledge would bring particularly valuable results. This is a policy which the Science Research Council has been pursuing in the past few years with success but also at the expense of the sensitivities of some research groups whose interests do not coincide with those of the research council. The Social Science Research Council seems to take the view that the nature of social science research is still so poorly understood that selectivity would be premature. But is this really so? Might it not indeed be sensible, at this early stage in the development of social science in Britain, to single out the areas in which growth seems at once feasible and likely and to hope that these will become intellectual bridgeheads from which a better understanding may be built out.

The risks inherent in the present policy of diverse support are quite considerable. To begin with, there is the possibility that the talents of good people may be scattered on too many unrelated projects, some of which are trivial. Second, there is the danger that no single centre of academic activity will be large enough to sustain the momentum of a successful research programme. Third, there is the certainty that many of the projects now begun will turn out, some years from now, to sustain research groups with whom the council will by then be disenchanted.

The council's hankering after research institutes of its own is another cause for anxiety, however strong the special reasons for setting up the Industrial Relations Research Unit and the Race Relations Research Unit (at Warwick and Bristol respectively). Why should not activities like these, interdisciplinary though they may be, fit into the more familiar structure of a university? The council may be right to say, in its latest report, that university departments are bad at collaboration with each other, but that is no reason to give up trying to beat their heads together. And the advantages of using the existing machinery of the universities for carrying out interdisciplinary research programmes are that teaching and research

are more intimately linked and that the bright trained people who emerge from the research programmes are likely to be scattered widely within the university system as a whole. Given that the research council's chief aim at this stage must be education, it is hard to think that the rewards of particular interdisciplinary

research programmes can be so great as to transcend the normal machinery of the universities. To be sure, the Social Science Research Council may say, with justice, that the two units so far established are well integrated into the system. Outsiders, however, may regard them as bad precedents.

Towards a Better Apocalypse

THE first report of Mr Russell Train's Council on Environmental Quality is a judicious mixture of tub-thumping, political savvy, popular science and good sense. It has something for almost everybody, and even a special message from President Nixon which says that the report "represents the first time in the history of nations that a people has paused, sociously and systematically, to take comprehensive stock of the quality of its surroundings" (see page 654). The President goes on to congratulate himself and his colleagues in the Administration for what has been done already to give the environment pride of place in the new scheme of things—the setting up of the Council on Environmental Quality was the first sign of the way in which the wind was blowing—but this makes the message not merely an exhortation but a reminder, valuable, no doubt, that even Mr Train's council is in the last resort a part of the Executive Branch and not an appendage of the Supreme Court. Its first report would have been more persuasive if it had not been so openly political.

To look on the good side, the report has nevertheless several sterling qualities that should not go unsung. It takes a broad view. There is, for example, a sensitive discussion of the myth that flits from one dinner table to another to the effect that economic growth as such is a potential hazard to the quality of life. The simplest form of the myth derives from two unconnected considerations—the belief that all power stations produce hot water (the cause of thermal pollution as it is called) in proportion to their output of electricity and the belief that electricity production is a monotonically increasing function of the GNP. So, the argument tends to go as the hand-dipped candles burn low, more GNP means more electricity and therefore more thermal pollution. Mr Train's report points to an elementary but nevertheless important flaw in the argument—that it is quite possible to increase the GNP of a nation such as the United States by making it compulsory to fit anti-pollution devices to the exhaust systems of automobiles, for the GNP, after all, is measured by the prices of manufactures and other goods. The only snag is that investment in better mufflers is unlikely to lead as certainly to future economic growth as investment in, say, power stations. The point is well worth making, for there is a great need to make sure that people cannot wriggle off the pin that compels a choice between one desirable thing and some other. Mr Train's report is also to be admired for what it has to say about the use of land in

the United States (and there may in the long run be great value in the way in which the President has endorsed this part of the report). What the council says is simply that the way in which local communities finance their general expenses by means of property taxes provides many of them with a vested interest in the urban sprawl. The moral, plain enough for everybody to see, is that there is an urgent need for centralizing the regulation of land usage.

All these are thoughtful matters. One of the defects of the council's report is that much of it is also trivial. The usual discussion of the dangers of accumulating carbon dioxide in the atmosphere is, it is true, hedged around with qualifications, among which the discovery that carbon dioxide is soluble in seawater shines through as if it had not been known all these years that the balance between the carbon content of the biosphere, the atmosphere, the oceans and the carbonaceous rocks is dynamic and not static. The same sense of wonder permeates the council's complaint that only one per cent of the money spent on meteorological research is spent on attempts to understand "man's inadvertent modification of weather and climate". So what? Is there any sense in seeking to understand the effects of, say, major afforestation schemes on climate in the surrounding region without knowing much more about the behaviour of the atmosphere on the mesoscale? And if there is need to understand more clearly the way in which a city can influence its own climate, is it not best to begin by seeking an understanding of atmospheric motion on an even smaller scale? In other words, the council runs a serious risk of putting the cart before the horse in what it has to say about meteorological research. And in this context, it may well have overlooked the importance of the political aspects of the problem (in the broadest sense), for it may well be that New York has become intolerable in the summer because one small air conditioning set is having to work flat out so as to process the exhaust from several others. The time may have come when cooperative air conditioning is the only feasible solution. Unhappily, the coordination of local policy necessary to bring about such a state of affairs, or to prevent municipal pollution of rivers or the foolish littering of the countryside, will require much more in the form of political organization than the institutions set up in Washington in the past few months as devices for persuading voters that something is now about to be done to safeguard the environment. The council

could have done better than to provide a blanket endorsement for these devices.

And with all its reflectiveness, it could have looked more deeply at the international implications of what it wants to say. It is true, of course, that Mr Train goes much further than previous sponsors of reports on the environment in drawing attention to the simple proposition that the environment is ultimately indivisible. Unhappily, this leads the council simply to a discussion of the importance of "monitoring the world" as one chapter heading has it. Why so few American organizations recognize the great indelicacy of this point of view will be a constant mystery to those outside North America who are sometimes painfully aware that only the richest nations can afford to fuss over the environment as the United States has been doing in the past few months. Mr Train should perhaps consider how it must sound to populations now graduating from bicycles to motor cars to be told that automobile exhausts are such a serious nuisance that their use should be severely curtailed. There is a

danger that to populations now struggling with a GNP per head which is hardly more than one per cent of that in the United States, well-meant introspection about the virtue of economic growth may seem a form of self indulgent smugness. And too much exhortation about the need for controlling the growth of the population may seem like neo-colonialism to nations in which the infant mortality rate is still mediaeval and in which a willing pair of hands is the next best thing to a machine. In short, if the environment is one global piece, is it possible to detach separate problems of air pollution from problems of foreign aid? And by this test, does the \$4,000 million that the Administration has set aside for the control of water pollution (most probably inadequately) make up for what it has agreed with Congress not to spend on the conditioning of the rest of the world to regard pollution as an evil and not a necessity? At one point in his report, Mr Train acknowledges that there is a long way to go in the fight for a better environment. It is even longer than he knows.

Swords into Rubbish

THE Department of Defense in the United States seems to have a nasty flair for mishandling its stocks of nerve gases. Not so long ago, large numbers of sheep in Utah were accidentally killed. Not so long ago, the state of Washington was up in arms (so to speak) because of proposals to store nerve gas there. Now there are protests about the scheme for sinking a ship laden with rockets which contain nerve gas off the edge of the Continental shelf off the south-east coast of the United States. The small communities along the two routes which will be taken by the two trainloads of disused rockets seem to be seized either with anger or indifference, but for many people the whole affair is too much an illustration of how the military, in their handling of potential hazards to the lives of people, can combine immense mechanical efficiency with a failure to reckon in advance with public opinion.

This is why it may be important first of all to acknowledge that the disposal of the rockets now under way is probably a simple consequence of the decision earlier this year that the United States would abandon the use of biological weapons and even back-pedal on the use of chemical weapons, nerve gases and the like. That, at least, is one point in its own defence which the Department of Defense might have advanced against those who have taken the movement of the useless rockets as yet another sign of the belligerence of the Pentagon.

It is also almost certain that the method of disposal now being used is safer than any other that might have been devised. If the nerve gas, supposed to be that known as GB, had been in simple containers, it would no doubt have been possible to dispose of it chemically, by hydrolysis in caustic soda. The difficulty in the present operation no doubt stems from the way in which

nerve gas and rocket fuel are held together in the same container. Given the precautions which are being taken along the routes to the coast, there is little cause to fear an overt accident. It is also probable that if the integrity of the concrete cases in which the rockets are contained should somehow be destroyed 1,600 feet below the sea, both the nerve gases and the rocket fuels would quickly be rendered impotent. (The half-life of GB gas in water is reckoned to be about 10 days, too long for disposal near the turbulent surface of the sea but probably just on the margins of acceptability at 1,600 feet.) This does not fully answer the question why the military have not been more ingenious in working out some way of denaturing nerve gas rockets without having to dump them in the sea.

So why is there all this fuss? The complaint against the Department of Defense is simply that it has once again been exceedingly cavalier in its regard for international proprieties. Especially at a time when the United States is taking a part of the initiative in the development of international law to govern the seabed (in contrast with the high seas, see page 656), it is almost beyond belief that there should be mounted such a tactless operation as the mass disposal of nerve gas. That even the British Government—by no means a steady opponent of United States foreign policy—should have sent three scientists off to make an independent assessment of such hazards as there may be off Bermuda should be a sign to the United States of how seriously the incident is regarded elsewhere. And if there should be any doubt, U Thant, the Secretary-General of the United Nations, should have left the planners in no serious doubt of the importance of what they plan.

The simple truth is that the care which has no doubt been taken to make sure that the operation will be

carried out safely does not in any sense absolve the United States Government from the need to discuss what it proposes fully with other nations which have expressed an interest. The fact that there is at present no legal requirement for the United States to seek permission in this way is quite beside the point—the best way to encourage the adoption of internationally acceptable restraints is to behave with restraint in advance of the formalities. The fact that the present operation merely repeats what has been done before is also irrelevant—a great deal of international experience in the past few years has only gone to show that precedents are false guides when the susceptibilities of other nations are concerned.

100 Years Ago



MR. DARWIN AND THE FRENCH INSTITUTE

THE judgment of foreign nations gives the best clue to that of posterity; and it is therefore with peculiar interest that the countrymen of Mr. Darwin have watched the reception of his works in France and Germany. In the latter country his theory of the origin of species has been more or less completely accepted by those best qualified to judge, including men like Gegenbaur and Haeckel; and it has produced a complete literature of arguments and facts "für Darwin," without encountering any very serious opposition. In France, the truth of the theory is far less extensively admitted, and has been lately the subject of prolonged discussion in the Academy of Sciences. The debate on Mr. Darwin's claims has now been adjourned for three months, but so far as it was reported in our last number it furnishes much ground for reflection.

At the present time, Imperial France is, perhaps, the most conservative in science of any country in Europe. It is not, therefore, surprising that, with a few exceptions like M. Claparède, French naturalists refuse to accept the theory of Natural Selection, and do not see (as others, and notably the Germans do) that it has already made a new epoch in human knowledge. Some, like M. Robin, object that it is not "demonstrable," and therefore not scientific at all; as if gravitation or the atomic theory had been, or could ever be, demonstrated like a proposition of Euclid. The Darwinian theory offers an explanation of acknowledged facts by the help of others equally indisputable, and it will only be "disproved" when it ceases to furnish an adequate explanation, or is superseded by a more simple and equally sufficient hypothesis. Meanwhile it fulfils, at any rate, one object of every theory, by stimulating research in all directions, and awakening new interests for the fresh investigations which it suggests.

From Nature, 2, 309, August 18, 1870.

OLD WORLD

RESEARCH COUNCILS

Social Science Emergent

THE annual report of the Social Science Research Council for the year ending on March 31 this year is a reflective document, containing not merely a record of how the council spent £2·27 million during the year (an increase of 65 per cent in a year) but also a thoughtful definition of the problems which arise in making research policy for the social sciences. To its credit, the council is the first to draw attention to the comparatively high cost of spending its money—£242,323 or close on 11 per cent of the total budget went on administration in the financial year just past. The council says that its comparatively high rejection rate (among research councils) is a part of the explanation, but there is also substance in the council's view that in present circumstances, there is bound to be a greater variety of opinion on the merits of particular research proposals than in other fields of inquiry. In practice, the council spent more than half of its budget on the support of postgraduate education in the social sciences, mostly in the form of studentships tenable at British universities. Research grants, as such, used up £0·86 million, compared with £0·58 million in the previous year.

The council's report says the open-ended character of social science research is no doubt part of the reason why potential applicants have found it hard to formulate convincing proposals for research projects. The council's goal is a research programme which promises a substantial advance of knowledge of "a connected series of social phenomena". Although the council rather wistfully regrets that much of its load of paper-work derives from the lack of scale and continuity which characterizes research in the social sciences, it acknowledges that a part of the trouble may be that social scientists are not yet as skilful as the natural scientists "in formulating viable research programmes". The council also says that new projects in the natural sciences can often be commended indirectly by the willingness of an existing institution to accommodate new work, and that the social sciences have not yet reached the point at which a substantial group of people in one place, possibly centred around equipment of common usefulness, can lend a pattern to the development of research.

On the development of criteria for deciding which projects to support, the council reaffirms its view that research grants cannot be distributed according to principles such as the urgency of the underlying issues of social policy. At the present stage in the development of the social sciences, the council says, nobody can say with certainty that a well designed piece of research will be irrelevant to the development of understanding in any discipline. The Social Science Research Council seems to have been encouraged in this view by the advice of the Science Research Council that the less radical policy of singling out areas of research within which work should be encouraged is not feasible so long as the difficulties and rewards of social science research are comparatively ill understood. That said, the council is not unwilling to single out particular research problems and has indeed, in

the year past, set up two research units in industrial relations and race relations.

One of the tendencies apparent in the council's report is a hankering after research units. The council says that the needs of interdisciplinary research are not always met within the structure of university departments and that "it is hard to avoid the impression that when any serious work needs to be done, there are powerful forces that drive the activity back into a series of separate departmental enclaves".

The 40 per cent of the SSRC budget spent on research grants was in 1969-70 awarded to 173 applicants, which implies a rejection rate of almost exactly 50 per cent and more money for fewer successful applications than in the previous year. The open-endedness of the social sciences as such is reflected in the way in which some of the council's money is spent on long-term open-ended research projects such as that under Professor J. Parry Lewis at the University of Manchester intended to devise a comprehensive urban simulation model. Support for postgraduate teaching is the most rapidly growing part of the council's budget, but the council is evidently prepared to look sceptically at the value of conventional PhD courses in the pattern of its activities.

LAW SUITS

Animal Research

THE settlement was announced this week of a libel action brought by Mr John Bleby, Director of the Laboratory Animals Centre of the Medical Research Council, against the weekly magazine *Titbits* and the British Union for the Abolition of Vivisection. In November 1968, the magazine apparently published an article about medical experiments with animals which included a photograph of Mr Bleby and allegations of cruelty to animals. The British Union for the Abolition of Vivisection was involved when it displayed in its premises in Whitehall an enlarged photocopy of the article. In the settlement out of court this week, *Titbits* and the British Union paid substantial damages to Mr Bleby and the union also agreed, in a statement in open court, to display in the windows of its Whitehall office a copy of the full report of the proceedings in court which is to be published in *Titbits*.

The chief function of the MRC's centre is to make animals more readily available to research laboratories, but it also gives advice on how to keep and house animals. One result of the centre's work is that by improving breeding techniques, inbred strains of animals can be bred more efficiently and fewer animals need to be bred for experiment than under a purely random system. Apart from research on breeding techniques, no medical research is carried out at the Laboratory Animals Centre.

SEISMOLOGY

England Shakes

LAST Sunday evening's earthquake which startled people in the northern counties of England would have gone unreported if it had occurred in one of the world's recognized earthquake danger areas. But by British standards with a magnitude on the Richter scale of

between 4.5 and 4.75, as estimated by the seismological unit of the Atomic Energy Authority, last Sunday's event rates as one of the largest to have been recorded. It was felt in Cumberland, County Durham, Westmorland, Lancashire and Yorkshire, and as *Nature* went to press the location of the epicentre had been narrowed down to the neighbourhood of the town of Kirkby Stephen on the Westmorland-Yorkshire border.

According to the geology department at the University of Durham, which makes a speciality of the study of British earthquakes, the earthquake on Sunday probably originated in either the Craven fault belt or the Dent fault, which form the southern and western boundaries respectively of the Askrigg block, named after the village of Askrigg in Wensleydale. The blocks in this area have been stable in themselves since the Carboniferous, of the order of 300 million years ago, so that faulting has tended to be concentrated at the boundaries. The Durham group are now looking up the records of an earthquake which geologists with long memories recall as having occurred in the same area in 1932.

Modern geologists will be pleased that the theory of continental drift and sea floor spreading can be implicated, although in a roundabout fashion. The likely explanation of Sunday's earthquake is that it was an adjustment of the crust related to the subsidence of the North Sea basin that has been going on since the Tertiary. It is then argued that the North Sea basin itself may be the result of the pressures that occur at continental margins as a result of sea floor spreading. An alternative explanation of the earthquake, less favoured at Durham, is that it was a dying whisper of the Alpine orogeny, the process responsible for Swiss scenery.

The depth at which the earthquake occurred is not yet known, but a quick look at the seismograph records suggests something like ten to fifteen kilometres. The surprisingly wide area over which tremors were felt suggests, however, a greater depth, possibly twenty kilometres, which places the earthquake only just within the base of the crust. When readings are received from stations outside Britain, it will be possible to fix the depth more precisely. A fault plane solution, giving the direction of movement that occurred, will be attempted at Durham. In the meantime, this earthquake was a nice reminder that Britain is not as seismologically quiet as people believe—in the past year or two there have been several earthquakes in the Bangor area with a magnitude of up to 4, probably associated with the faulting along the Menai Straits.

CHEMISTRY

Removing the Boredom

by our Education Correspondent

CHEMISTRY students at the University of Sussex may take part next session in trials of a scheme designed to make undergraduate chemistry courses more attractive and less boring. The scheme, outlined by Professor Colin Eaborn in the current issue of *Chemistry in Britain*, will be run alongside conventional chemistry courses. Its chief aim is to take some emphasis away from formal lecture courses and to base the degree on creative research work.

Professor Eaborn, who is chairman of the science faculty board at the University of Sussex, points out

that the demand for places in chemistry departments of many British universities has been falling steadily during the past few years, and most universities now admit students who achieve only the minimum entry qualifications. "It is my impression," Professor Eaborn says, "that many students, especially those with the more lively minds, find chemistry courses boring. I suspect that the major defect of existing courses is that they give the student little or no opportunity to exercise his originality, individuality and creative ability."

What Professor Eaborn suggests is that during the first or second year of a chemistry course, an undergraduate should be given a research problem which will be his chief concern from then on. At the same time, he should familiarize himself with the usual range of chemical knowledge either through existing lecture courses or through suitably modified courses. The research topics should be chosen according to two basic criteria: there should be a good chance that hard work will lead to results, and the research should draw on wide areas of chemistry.

As far as assessment is concerned, Professor Eaborn believes that in addition to a final thesis, there should be taken into account a student's approach to his work, as shown by literature surveys and essays directly or indirectly related to his project. "If we could produce a distinctly better graduate by abandoning assessment altogether I believe we should be prepared to do so," Professor Eaborn suggests, "but my own view is that degree classes based on the course I propose would be at least as accurate as the present examination grades as indicators of future achievement."

The chief attraction of such a course is that an undergraduate would be motivated to learn by his own reading, which would be stimulated by enthusiasm for his research activity. But the chief drawback is the cost in terms of teaching time and laboratory equipment. Calculations carried out at Sussex suggest that an undergraduate might cost between one-third and one-half as much a year as a research student. Professor Eaborn points out, however, that university plans for the 1972-77 quinquennium must be formulated during the next twelve months or so, and that this is a good time for chemists to seek additional funds "for a bold scheme which might transform the attractions of chemistry as a university subject".

The chemistry faculty at the University of Sussex has decided to try the scheme out on a few students next session, but final approval must first be obtained from the Senate.

COMPUTER SCIENCES

Quickening Pace in Software

A NEW recruit to the band of software companies was recorded last week with the decision of GEC-Elliott Automation to set up its own subsidiary organization. This follows only months after the creation by ICL of a separate software company, Dataskill, and adds further weight to the notion that the software business has already overtaken the hardware side as the chief money spinner in computing.

It seems that the shortage of trained programmers, however, played an important part in convincing GEC-Elliott that the time was ripe to commit resources to a new company—its first new systems company since

the formation of the group nearly two years ago. One innovation will be a system of data terminals on line to the computer centre through which it is hoped that women confined to the home will be able to continue their work as programmers. The company will have two large computers at its disposal and will, to begin with, operate with 40 or 50 people.

The latest figures from ICL indicate that expenditure on software is increasing by something like 25 per cent a year. It is estimated now that more than 40 per cent of the total expenditure on computing in Britain is swallowed by up software, which amounts to a national turnover of over £200 million. One of the complaints of the much lamented Select Committee of the House of Commons was that government departments are reluctant to make efficient use of the facilities of software houses because of the requirement that they must make full use of their own permanent staff.

Although the Select Committee was disbanded in June, Mr Eric Moonman and two of his fellow ex-MPs are determined, it seems, that the hard work of the subcommittee investigating the computer industry should not go by default. They intend to publish an account of what the committee would have reported to parliament had Mr Wilson held back the general election until next year. This will take the form of a collection of articles combined into two separate volumes. Mr Moonman will be writing about the international posture of the British computing industry, Mr Eric Lubbock will be concerned with the government's purchasing policy for hardware and Mr Arnold Gregory will write about the impact of computers on education. The three non-parliamentarians involved are Professor S. Gill of Imperial College, London, who will contribute on the impact of computers on telecommunications, Professor A. Douglas of the London School of Economics who will write about the government's purchasing policy for software and Mr F. Lamb, also of LSE, who will contribute on the government's support for research and development.

REGIONAL MUSEUMS

University Takeover

THE Swansea Museum, once described by Dylan Thomas as a museum that ought to be in a museum, seems now to be well on the way to oblivion under the shadow of the University College of Swansea. At a meeting at the Royal Institution of South Wales at the end of July, the membership of the institution and their proxies turned out to be in favour of a proposal that the University College should in future pay for the cost of the upkeep of the museum and that, with luck and at the beginning of the new university quinquennium, the university might provide extra staff. At the meeting at which this proposal was accepted, several members appear to have spoken against the partnership with the university, some on the grounds that not sufficient effort has been made to preserve the independence of the museum and that, in any case, if there has to be a benefactor, it should be the Corporation of Swansea and not the university.

The new arrangement will give the university the lease of the museum, but the university agrees "for as long as the Royal Institution continues in existence" to set aside some space "such as a room or part of a room" for the administration of the institution.

The future of the museum which the Royal Institution of South Wales is best known for is not entirely clear from the resolution accepted by the membership in July, but it does appear that there will be a joint management committee and that the university may in due course appoint a director. Like many similar institutions, the Swansea museum has suffered since the Second World War from the discovery that its once handsome endowment is now hardly sufficient to pay for electric light and heating. In successive attempts at modernization, the museum has indeed been made to seem less interesting. The banishment of the stuffed elephant that used to guard the doorway was no doubt an acknowledgment of the need for modernization, but it seems not to have been followed by a hardheaded appraisal of what the function of a museum might be in a modern industrial city.

WATER RESOURCES

Water for Wales

THE control of water resources in Wales is still too fragmented in spite of some recent reorganization, and the authorities involved take too narrow a view of their responsibilities, according to the Welsh Council. In its advice to the Secretary of State for Wales (*Report on Water in Wales*, available from the Welsh Council, Cardiff), the council complains, for example, that the river authorities lack positive powers to advance the wellbeing of areas in which reservoirs are built. A more coordinated approach could be taken instead by a new body, a Water Development Authority for Wales, which should have wider responsibilities than the existing river authorities and the Water Resources Board to build and regulate reservoirs and to develop their use for recreation. The authority could be financed, the council suggests, by levies on water supplied to the various water undertakings, and, being a commercial organization, it should be able to deal more satisfactorily with the often tricky negotiations for compensation for land acquired for reservoirs.

The council does not consider desalination a practical alternative to conservation and storage of river water, but points out the advantages of estuary storage schemes which, it believes, are less likely to raise objections in Wales than are upland reservoirs. It asks for the early completion of studies on the feasibility of the Dee Estuary for water storage, and also for the initiation of a study into possible storage schemes in the Severn Estuary and elsewhere. The council points out, however, that estuary storage is unlikely to make a contribution until the end of the 1970s, and even then, some upland reservoirs will still be needed.

Reservoirs in rural areas in Wales could, the council says, be made profitable to the communities if they were properly developed for recreation. The council also emphasizes the need for careful siting of buildings, and it comments that some of the reservoirs recently built in Wales might have fitted more satisfactorily into their surroundings if the dams had not been of stark white concrete.

The government will now consider this report alongside the forthcoming regional study from the Water Resources Board and reports which are due from the Central Advisory Water Committee and from the Sports Council.

ANIMAL HEALTH

Cows Not Well

THE first incidence of acariasis in bees was reported in Romania last year, and colibacillosis of pigs was confirmed in Fiji. Camels in Somalia were suspected to have anaplasmosis, but bluetongue was no longer recorded in India. Once more the health of the world's animals has been analysed, tabulated and published by the Food and Agriculture Organization as the *Animal Health Yearbook*. The information about foot and mouth disease shown here is part of a more comprehensive table depicting the incidence of the disease in Europe, together with each country's vaccination policy. From South America, a success story is recorded in Argentina, where vaccination has contributed to the establishment of the area south of parallel 42 as a disease free zone. Less happily, throughout the continent last year several new sub-types of foot and mouth virus were reported.

OUTBREAKS OF FOOT AND MOUTH DISEASE IN EUROPE IN 1969

	Outbreaks in												Vaccination					
	J	F	M	A	M	J	J	A	S	O	N	D	Total	C	P	C	V	P
United Kingdom (Great Britain)													0					+
United Kingdom (N. Ireland)													0					+
Iceland													0					
Denmark												+	8			+		
Norway													0				+	+
Sweden													0				+	+
Finland													0					+
Netherlands													0	+	+			
Belgium												+	3	+	+			
Luxembourg													0	+	+	+		
France	+	+	+	+	+							+	35	+	+	+		
Fed. Rep. Germany			+	+									12	+	+			
Switzerland						+							1	+	+			
Austria*													0					+
Italy	+	+	+	+		+				+	+	+	132	+	+	+		+
Malta													0					+
Spain	+	+	+	+	+	+	+	+	+	+	+	+	522	+	+	+		+
Portugal	+	+	+	+	+	+	+	+	+	+	+	+	160	+	+	+		+
USSR	+	+	+	+	+	+	+	+	+	+	+	+	473	+	+	+		+
Poland				+	+	+							6			+		
German Dem. Rep.										+		+	4	+				
Czechoslovakia	+	+	+				+		+			+	7	+	+			
Hungary													0	+				
Romania	+												6			+		
Bulgaria													0					+
Yugoslavia													0					
Albania													0					
Greece	+	+		+					+	+	+	+	111			+		
Turkey	+	+	+	+	+	+	+	+	+	+	+	+	1,654	+	+	+		+
Cyprus													0					
Israel	+	+	+	+	+	+	+	+	+	+	+	+	24					
	J	F	M	A	M	J	J	A	S	O	N	D	Total	C	P	C	V	P

Vaccination may be CP, compulsory periodically; CO, compulsory in outbreak areas; V, voluntary; and P, prohibited.

* In Austria vaccination is prohibited unless officially ordered.

HUMAN ECOLOGY

Man under the Microscope

MAN's relationship with his environment, in all its multidisciplinary glory, has been taken under the wing of the Commonwealth Human Ecology Council (CHEC), which was set up officially last November. It has come into the limelight with the announcement of plans for a Commonwealth conference on development and human ecology. Joint sponsorship of this conference, to be held in Malta next October, is the first major project of the council, which has the ambitious aim of seeking ways to encourage Commonwealth governments to avoid the mistakes of the past when planning future developments—agricultural, medical, economic or whatever.

This wide ranging aim is reflected in the composition of the executive committee, which includes Dr E. B. Worthington of the International Biological Pro-

gramme. Professor H. Bowen-Jones, director of the Centre for Middle Eastern and Islamic Studies at Durham, and Mr J. Owen Jones, director of the Commonwealth Bureau of Agricultural Economics. The chairman is Mr A. S. H. Kemp, secretary general of the Royal Commonwealth Society, exemplifying the belief that the Commonwealth is a valuable unit in which to pursue the study of human ecology.

CHEC evolved from a committee on human nutrition in the Commonwealth, which, in the 1950s and early 1960s, had such active members as Professors B. S. Platt and R. A. McCance looking for a closer relationship between agriculture, medicine, nutrition and education. This committee conceived the idea of a pilot case study of a small Commonwealth region so that hard facts could be established on an ecological basis. From this idea grew a study of Malta, which the Government and Royal University of the island began in 1966. To complement the activities in Malta with expertise and advice CHEC came into being as a non-profit-making company.

So far the medical side of the Malta project has involved a survey of the physical development of children with respect to nutrition, and a survey of attitudes to health in rural communities. A socio-economic survey is under way, and at the planning stage are surveys of soil and water balance needs, agricultural and food resources, education and physical planning and urbanization.

CHEC hopes that the findings of this and future projects—none are at present in the pipeline—will not only help planners in the regions concerned, but will also be applicable to similar regions in developing areas of the world such as Africa and India. CHEC and the other bodies which are supporting its activities—Unesco among them—feel that this could be a way of providing guidelines for development in harmony with the environment. The first fruits of these grand designs will be made known at the conference in October. With the experience of the Malta project in mind, it has the object of “examining problems of satisfying essential human needs while establishing an ecologically sound balance between man and his environment”.

ASSOCIATIONS

New Image for ANZAAS

SOCIAL responsibility is rapidly becoming a catchword among scientists in Australia and New Zealand. Not only has the Australia and New Zealand Association for the Advancement of Science adopted the aims of the social responsibility movement and embodied them in its new constitution, but the association's new-style magazine *Search* is anxious to publish articles concerned with the social and economic consequences of science and technology.

The new constitution of ANZAAS, which was proposed last August by a committee under the chairmanship of Sir John Crawford, is essentially a response to the growing dilemma which faces many such organizations. Specialized societies have steadily been taking over the association's role of bringing together scientists for the exchange of information and news, and ANZAAS was forced either to change its aims and functions or to do nothing and fade into obscurity. The aims set out in the association's new

constitution deftly solve the problem of bringing its members together to pursue a common cause—to ensure that science and technology are used for social good—and at the same time they should help to promote science in an atmosphere which is becoming increasingly hostile to scientific development.

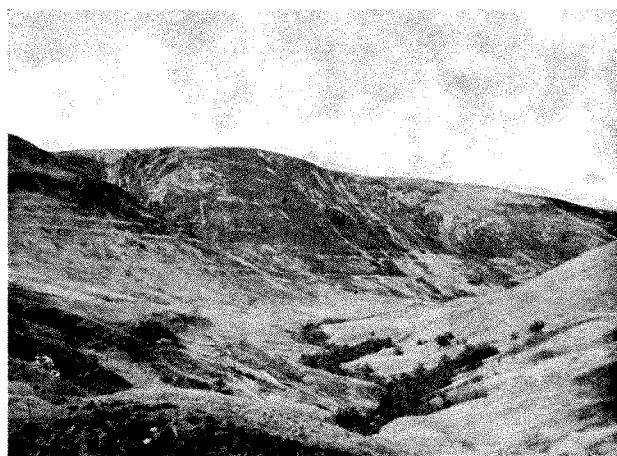
The aims of ANZAAS, as stated in the new constitution, are “the advancement of knowledge by bringing together scientists of all types and occupations so that they may interchange information and ideas, and making the public aware of what science is, what scientists do, and of the applications and implications of scientific discoveries to their everyday affairs and those of the nation”.

Sir John's committee clearly saw the need for improved communications between members of ANZAAS for carrying out the new aims of the association, and it took a hard look at the ANZAAS journal, the *Australian Journal of Science*. The first thing to be done, the committee decided, is to give the magazine more money, and to make it more topical and outward looking. The results were seen recently with the publication of *Search*, the new magazine of ANZAAS which has a completely different editorial policy from the old journal.

Mr J. B. Davenport, the magazine's editor, explained in an editorial that *Search* intends to publish review articles which should be comprehensible to scientists not working in that particular field, articles which deal with the social and economic consequences of science and technology, news and comment, and short research communications from scientists working in Australia and New Zealand. Science education will probably receive considerable attention in the magazine's pages, and the first issue contains articles on universities and research and the place of science in Australian education. *Search* is certainly a refreshing change from the *Australian Journal of Science*.

NATURE RESERVES

Mementoes of the Ice Age



A section of the famous “parallel roads” in Glen Roy near Fort William in Inverness, an area which has recently been purchased from the Forestry Commission and subsequently declared a national nature reserve by the Nature Conservancy. The three “roads” or terraces which run along the sides of the glen mark the former water levels of lakes which had been dammed by glaciers during the Ice Age. The highest terrace is at a height of 1,155 feet above sea level, the middle at 1,077 feet, and the lowest at 862 feet.

NEW WORLD

Congress Deaf to Prophecies

"THAT we are in a budgetary crisis no one can doubt; nor can one doubt that the crisis was foreseen; and that we did little five years ago when the crisis was predicted precisely in the National Academy of Sciences' report to your Subcommittee." With appropriate expressions of regret Dr Alvin Weinberg, director of the Oak Ridge National Laboratory, assumed last week the not wholly unpleasurable role of Cassandra before the House Subcommittee on Science, Research and Development, which has now completed four weeks of hearings on the issue of whether the United States should develop a national science policy.

In a brief but forceful statement, Dr Weinberg reminded his listeners of the forecast made in 1965 by the NAS committee, of which he was a member, that the basic physical sciences would face a major funding crisis because the agencies that had accepted responsibility for them were not at that time being permitted to expand. The committee had also recommended that the National Science Foundation should become a billion dollar agency by 1970. "It is little consolation to this subcommittee and its distinguished chairman (Mr E. Q. Daddario) to have foreseen as they did in the 1965 hearings the present predicament of science, and yet not to have been able to implement the major recommendation of (the NAS committee) greatly to expand the National Science Foundation." Dr Weinberg added that in his view the expansion of the foundation was the most important single action to be taken and that if the subcommittee's hearings resulted in nothing else than achieving for the foundation a level of support equivalent to one-tenth of a per cent of the gross national product, or about \$1,000 million a year, he believed that they would have been brilliantly successful.

This reflexion evoked a notable disquisition on the limits of the subcommittee's power and the impotence that has tempered its nevertheless substantial record of achievement. Mr Charles A. Mosher, a Republican member of the subcommittee, observed that although in his opinion the committee had done an extremely important job over the years, "I don't see that it has had much impact on the rest of Congress or on the Congressional decisions, particularly those taken by the Appropriations committees. . . . Too often, it seems to me that the Appropriations committees [which grant the actual monies up to a maximum limit of, but often less than, the sum approved by the Authorization Committees] and the Authorization committees don't have any coordination or even communication. . . . I think it is a sort of mistake that we did as well as we did".

Referring to Dr Weinberg's statement that the subcommittee had heard predictions in 1965 of the present crisis, Mr Mosher continued, "We pointed to it, but who listened? I don't think there is the machinery by which our considerations in this committee are known

by more than a few people. I think the only [sc. effective] devices are really informal devices and the chance of a very vigorous personality. Now Mr Daddario, our chairman, is that type of personality and he has some personal impact. I don't think that the machinery itself, the legislative process itself, is effective or efficient in this regard. I regret to say it. I think it is crucial to the considerations we are discussing".

Mr John W. Davis, presiding over the subcommittee in Mr Daddario's absence, suggested that at least the authorization committees helped by preparing witnesses to make a better showing before the appropriations committees. Dr Weinberg, declining to comment on the relative influence of the two types of Congressional committees, at least agreed on the importance of the charismatic figures such as the late Representative Fogarty who persuaded Congress to keep the money flowing into the National Institutes of Health. Dr Weinberg went on to express his "highest regard for the new director of the National Science Foundation, Dr McElroy: I think he is going to be awfully, awfully good because he has that kind of public presence and dedication . . . I hope he is the one who succeeds in putting across the suggestions that are in here and were in the committee report five years ago".

Like several previous witnesses, Dr Weinberg

CANCER RESEARCH

New Rationale

"SUPPOSE we could develop a vaccine that would immunize us against cancer, say with the same certainty as the Sabin vaccine immunizes against polio. . . . Now an important source (though by no means all) of our concern about environmental pollutants, particularly radioactivity, is prompted by the belief that these agents, even in tiny amounts, are carcinogenic. To prove or disprove their carcinogenic potential at the very low levels at which they appear in the environment is impossible. It seems to me that a more rewarding strategy would be to develop antidotes for the possible untoward medical sequelae of these pollutants. Of these sequelae, cancer is the most important. If cancer could be eliminated by vaccination, our whole attitude toward many environmental problems would change from panic to reason. Thus my plea for heavier support of the National Cancer Institute, and other biomedical research, is entirely consistent with the shift in emphasis toward the environment."—Statement by Dr Alvin Weinberg to the House Subcommittee on Science, Research, and Development.

expressed his distaste for what he saw as the anti-scientism that "is now growing and that could eventually destroy science". Dr Weinberg could only say that this counter culture was "dead wrong" and that its raucous complaints, "which echo in the universities and complicate the relation between the Government and the scientific university, are to be satisfied by more and better science, not by less science".

Equally grave perils were described by another witness before the subcommittee last week, Dr Harrison Shull, Dean of the graduate school of Indiana University. Dr Shull began by saying that the post-war science policy "is now clearly in a state of chaos and perhaps impending collapse" but proceeded to offer some ingenious guidelines for correcting the balance. The annual appropriation cycle is not a suitable way of financing the longer term needs of basic research which, Dr Shull suggested, follow a natural time cycle "of the order of twice the time span of one student's graduate education", or about six to ten years. Congress should provide appropriation mechanisms to ensure such long-term continuity.

Dr Shull criticized the regional distribution of the science fellowships administered by the National Science Foundation. In the financial year that ended

in June 1969, more than half of the 2,500 fellowships in the foundation's gift went to only six institutions. How many institutions should receive so high a level of support? This is the job of a national science policy to decide, but Dr Shull noted that since the 5 million souls of Indiana support three first-rate institutions, the 20 million of California some 11 such bodies, and the 55 million of Britain 27, there is the indication that between 1.5 to 2 million people provide an appropriate tax base and population base to support one first-rate advanced institution. On this reckoning there should be about a hundred such institutions in the United States, well distributed geographically.

Another concern of a national science policy should be manpower planning and Dr Shull believed that the present surplus of PhDs on the job market should have been predicted and forestalled. Dr Shull committed himself to the prediction that there will be an equally severe shortage of PhDs in the hard sciences in five years' time, because the federal government "has reacted so abruptly in changing its support pattern, and there has been so much adverse publicity discouraging young people from entering graduate school and encouraging university departments to limit enrolments artificially".

Literacy for Polluters

"OUR environmental problems are very serious, indeed urgent, but they do not justify either panic or hysteria. The problems are highly complex, and their resolution will require rational, systematic approaches, hard work and patience." These were the words with which President Nixon sent to the Congress this week the first annual report of the Council on Environmental Quality, a document that is to serve as the order of battle for the coming year's campaign against pollution and beyond that to educate the public and polluters into environmental literacy.

The report* represents the first attempt in the United States, and probably in any other country, to consider the whole range of points at which the environment has been altered by man to their mutual detriment. For the benefit of the layman it describes in simple but exact language the various types of pollution that befoul the air and waters and suggests how each should be combated. Many of the fifty or so specific recommendations made in the report have already been embodied in legislative proposals sent to Congress earlier this year which are still awaiting decision.

The Council has not been content to keep its nose to smog, sludge and oil slicks but has lifted its eyes to include the pressures of population on land and on scarce natural resources, and from there to consider the more fundamental question of whether economic growth should be restrained for the sake of conserving the environment. This perhaps is the crucial point at which the ecological fetishists part company from the

rest. The Council, in its own words, has taken "a broad view of the term environment and this broader view reveals many benefits of continued economic growth. Our society needs more goods and services of many kinds—better housing, improved public transportation, more adequate facilities for health and education, and increased pollution control. It is likely that funds for such investments will come not from a cutback in the production of cosmetics, for example, but from an overall increase in national output. Moreover, a reduction in growth would result in a severe blow to the aspirations of the economically disadvantaged, especially minority groups".

This is not to say that the economy can remain unaffected by the attempts to preserve the environment. The Council laconically remarks that in the long run the control of water pollution may possibly require "some changes in the products that people consume . . . and will lead to higher prices for some products". A major theme of the report is that pollution is at present being encouraged by misplaced economic incentives, in that the cost of preventing pollution is borne by the community downstream or downwind of the factory instead of by the manufacturer and his customers. These "external social costs" should be reflected in the price system. Another type of misplaced incentive is the property taxes which lead to bad land use by encouraging local governments to favour industrial developments against houses, which cost more in terms of the schools and hospitals that must accompany them than they yield in property taxes.

The keynote of the Council's report is not despair

*Environmental Quality, the First Annual Report of the Council on Environmental Quality, US Government Printing Office, Washington 20402. \$1.75.

Table 1. ESTIMATED FEDERAL FUNDING FOR POLLUTION CONTROL AND ABATEMENT, BY AGENCY AND BY POLLUTED MEDIUM AND SELECTED POLLUTANTS (FISCAL YEAR 1970 OBLIGATIONS) FROM THE REPORT OF THE COUNCIL ON ENVIRONMENTAL QUALITY.

(In millions of dollars)

Agency	Polluted medium			Selected pollutants ¹						Total
	Air	Water	Land	Pesti- cides	Radia- tion	Solid wastes	Noise	Ther- mal	Other	
Interior	4.0	629.9	2.6	5.2	0.2	5.7	—	2.4	—	650.0
AEC	4.6	5.8	0.2	0.1	133.3	2.1	—	1.8	—	147.9
HEW	94.2	3.5	—	11.7	18.6	15.2	0.1	—	7.9	151.2
DOD-military	19.9	35.5	—	0.7	0.8	0.2	12.5	—	0.1	69.7
Agriculture	12.0	120.7	7.9	23.2	0.7	2.6	—	—	0.4	167.5
Transportation	5.8	6.0	—	0.2	—	0.4	7.3	—	—	19.7
NASA	2.3	0.9	—	—	—	0.1	14.0	—	—	17.3
Appalachian Regional Commission	—	4.6	6.5	—	—	1.0	—	—	—	12.1
TVA	14.5	10.2	0.1	—	1.4	0.1	—	6.8	—	33.1
Corps of Engineers	0.4	3.0	—	—	—	1.8	—	—	—	5.2
Commerce	1.7	3.4	0.2	—	0.1	0.2	0.2	—	0.4	6.2
Justice	0.3	—	—	—	—	0.9	—	—	—	1.2
Other	3.5	5.0	2	0.1	0.4	0.4	—	—	0.4	9.8
Total	163.2	828.5	17.7	41.2	155.3	30.0	34.1	11.0	9.2	1,290.9

¹ Excluding funds reported in media columns.

Source: Compiled from data supplied by the Office of Management and Budget, July 1970.

but more what company chairmen invariably describe to stockholders as "cautious optimism"—in other words, a feeling that matters do not necessarily have to progress from bad to worse. What the Council's recommendations boil down to is first and foremost that Congress should not drag its feet with the substantial volume of legislative proposals on the environment that are already before it. These include the setting up of an Environmental Protection Agency to gather under its wings the bits and pieces of environmental control developed by other agencies and the National Oceanic and Atmospheric Administration which would do the same for oceanography and meteorology. Besides the strengthening of the institutional structure for protecting the environment, the Council also makes numerous proposals by which changes in the environment—such as mercury pollution of lakes, for example—will be spotted not too late in the day.

The meat of the report is a description which verges between the scatological and the eschatological of the damage done and the problems to be faced. Only a third of the population of the United States is served by an adequate sewage system and another third is served by no system at all. By 1980 one fifth of the total fresh water run-off of the country will be required for cooling operations by the electric power industry. The refuse collected for each town dweller in the United States has grown from 2.75 lb a day in 1920 to 5 lb a day in 1970, and will reach 8 lb in ten years time. In 1969 Americans threw away about 250 million tons of solid wastes, of which about three-quarters was collected and the rest junked. The collected wastes included 30 million tons of paper, 4 million tons of plastic, 100 million tyres, 30,000 million bottles, and 60,000 million cans. Among the uncollected trash is some 15 per cent of the 9 million cars abandoned each year, and between 1,000 to 2,000 million bottles and cans. There are prospects for recycling some of this litter, if it could be collected; almost the only direct use the Council has come up with is for automobile tyres which, it is suggested, could be used to make artificial reefs for fish breeding along the flat ocean bottom off the Atlantic coast.

The Council's report is more than a month overdue,

though this is hardly surprising since despite the report's appellation as annual, the three members of the Council were not appointed until January this year and have had less than four months in which to survey the state of the nation's environmental health. In these circumstances it is something of an achievement to have produced a report at all, let alone the synoptic and reflective document that has now appeared. The Council is chaired by Mr Russell E. Train, formerly Under Secretary of the Interior and from 1965 to 1969 president of the Conservation Foundation. Of Mr Train's two colleagues, Mr Robert Cahn is on leave from the *Christian Science Monitor*, having won the Pulitzer Prize in 1969 for articles on the national parks, and Dr Gordon J. F. MacDonald is a physicist (one of his interests is the mathematical treatment of the orbital history of the Moon) who has served on several government scientific committees including the President's Scientific Advisory Committee. The Council is served by a professional staff of 15 people, of whom the senior scientist is Dr Lee M. Talbot, previously an ecologist with the Smithsonian Institution.

In the coming weeks the Council's report will be examined before several Congressional committees and as a compendium of facts and recommendations about the environment it will doubtless command considerable attention both within and beyond the borders of the United States. Its lucid prose at least entitles it to a wide readership and if it gains that it will have served one of its principal purposes which is simply to educate.

SST

Booms in the Stratosphere

THE project to build a supersonic transport has run into renewed complaints from the environmentalists. Though the SST may break through this particular sound barrier, the chances of it passing unscathed through the impending Senate hearings on its fat \$290 million budget are probably less bright than at any time in the past.

The SST was one of the topics discussed at the Study of Critical Environmental Problems, a month long conference sponsored by the Massachusetts Institute of Technology and attended by an influential group of scientists from government, industry and the universities. Reporting their conclusions earlier this month, the group expressed concern lest large scale operation of SSTs should begin before the effect of the aircraft on the stratosphere and global climate was better understood. The recommendations of the group were, in brief, that the combustion products of the SST's engines should be better characterized so as to estimate how much water vapour and particulate matter will be injected into the stratosphere by the aircraft, and how these changes are likely to affect weather and climate.

Another attack on the SST has been pressed by the Environmental Defense Fund, which has already feathered its cap by getting the haul road for the Alaska pipeline delayed and requiring the Department of Agriculture to justify its continued use of DDT. The Fund has now taken court action to order the Federal Aviation Administration to set noise and other standards for the SST before granting a certificate of airworthiness.

The resourceful proponents of the SST in the government have met these criticisms by acknowledging the legitimacy of the concern expressed and setting up two councils. Mr William M. Magruder, an aeronautical engineer who since April has headed the SST programme in the Department of Transportation, announced last month that an Environmental Advisory Council would be set up under Dr Myron Tribus, an atmospheric physicist who is Assistant Secretary of Commerce for Science and Technology. This council will study the atmospheric problems raised by the SST, while an equally prestigious Noise Advisory Council, headed by the acoustical engineer Dr Leo Beranek of Bolt, Beranek and Newman Inc. will look into the decibels involved. Mr Magruder also announced that a research programme costing \$27.6 million over three to four years would be carried out by the Naval Research Laboratory and other outside agencies.

Mr Magruder's office is confident that the necessary technological solutions can be found to cope with the SST's contamination of the stratosphere. The high operating temperatures of the General Electric engines being built for the Boeing SST ensure that little hydrocarbon soot is produced. Particles derived from sulphur dioxide are a possible serious source of contamination but it seems more than likely that the sulphur content of the aircraft's fuel can be reduced without significant increases in cost.

The MIT group thought it unlikely that the carbon dioxide introduced into the stratosphere could affect the heat balance, or that the water vapour would reduce the amount of ozone—two possibilities that have been much in the air. But an increase in water vapour of only a few parts per million would amount to a global 10 per cent change which would be expected to increase cloudiness and perhaps to affect climate. The finding that water vapour in the stratosphere above Washington has increased by 50 per cent since 1964 is evidence of how much remains to be understood about this rarefied region.

At \$290 million, the SST's budget for the present financial year is more than twice the size of last year's

appropriation and the plane's neck will offer a tempting target for the Congressional axe. The budget has already been approved by the House of Representatives, although by a much narrower margin than in previous years, and it is unlikely to enjoy plain sailing through the Senate. Even if the American SST should falter at this or a later hurdle, the research programmes now under way are unlikely to be called off as long as the Concorde and the TU 144 threaten to live.

OCEAN DUMPING

Seabed Legislation

from a Correspondent

PLANS for internationalizing the deep seabed and exploiting it for the benefit of mankind as a whole go on apace, but not quite fast enough to prevent the unilateral dumping of dangerous nerve gases in international waters off the Bahamas. This happening would be illegal, it seems, under any of the draft conventions under discussion at Geneva in the current month-long session of the UN Committee on the Peaceful Uses of the Seabed. The discussions intended to lead eventually to an international treaty with the objectives of (1) banning all aggressive military uses such as missile silos; (2) the precise definition of an International Seabed Zone under some kind of international jurisdiction; (3) exploitation of this area for the equal benefit of all nations irrespective of their technological competence and geography (such as coastless countries and those without the resources to bore for minerals a mile or miles below the surface); and (4) protecting the area from harmful environmental interference and for free scientific investigation.

Three countries have tabled draft conventions at this session. By far the most comprehensive is that of the United States Government following President Nixon's directive of May 23 this year. Both France and Britain have produced partial drafts for discussion. The principal differences relate to the definition of the proposed International Zone of the seabed area and to the mode whereby the have-not nations are to obtain revenue from the exploitation undertaken by the haves. The US proposal comes out firmly for making the boundary between "international seabed" and that under some kind of control its 200 metre contour. This, of course, generally corresponds with the edge of the continental shelf. The same draft also proposes an extension of territorial waters out to 12 miles, and a trusteeship role for the seaboard countries over the exploitation of the international region beyond their areas of "national jurisdiction". (Such countries would be entitled to between 30 and 50 per cent of the profit from the exploitation activities, with the rest going to a kind of international kitty for the non-exploiting countries.)

The question of how to apportion the contracts and their proceeds is likely to provoke the most debate. The Soviet Union has already made its objections to any idea that corporations as distinct from sovereign states should be entitled to do the exploiting. Britain has a simpler plan specifying only two classes of seabed zone—territorial and international—and with exploitation licences for international distribution and royalties, collected by the exploiters. Under any agreement, there will have to be provision for the dumping of waste.

Company for Comsat

from our New York Correspondent

THE communications satellite corporation (Comsat) and AT & T will soon face stiff competition from other companies anxious to break their monopoly in domestic long-distance communications. There are not yet any communications satellites covering the United States, and all companies in this field must lease lines from AT & T. Until recently, it was assumed that the Federal Communications Commission would automatically grant Comsat the right to launch domestic satellites. But on March 24 the FCC invited filings from all "financially qualified companies", and last Friday announced that all companies interested in launching a domestic satellite system must apply by August 19.

Western Union is the only company already to have submitted a proposal, but others, including Comsat, are expected to follow in quick succession to meet the August 19 deadline. At this time, the other competitors are expected to include a consortium of the three major broadcasting networks, (NCB, CBS and ABC), the TelePrompter Corporation (a cable television company), General Telephone and Electric Corporation and the Data Transmission Company, a subsidiary of the University Computing Company. All contemplate a working system within two to three years after FCC approval.

There are at present only a limited number of optimum orbital parking spaces in which geosynchronous satellites can efficiently cover the entire United States without interfering with each other, but the number may increase as technology improves and if a broader bandwidth is approved by the FCC. At present, however, the FCC is unlikely to authorize a number of simultaneous systems. A spokesman for the FCC stated last week that no decision will be made until all applications are in. The FCC must then decide, he said, if it will approve only one system, several competing systems or several complementary systems, including both the satellites themselves and the Earth stations which must receive and transmit signals.

Western Union filed a proposal early last week to put up three geosynchronous satellites, two working and one back-up, with a total capacity of 9,000 channels and ten video channels. (A video channel is the equivalent of 200 audio channels.) The system would also include six Earth stations to receive the signals and 31 microwave relay stations to link the ground system throughout the country. The initial cost would be in the range of \$95 million with a running cost of \$10 million, but Western Union estimates that once the entire system was in operation the yearly revenue would be \$28 million.

Most of the other plans under consideration will probably be more complex. Page Communications Engineers in Washington, DC, has just completed a feasibility study for the networks, and has concluded that either a satellite system or a ground microwave system to carry radio and television signals for NBC, CBS, ABC, and the Corporation for Public Broadcasting, the educational network, would cost from \$50 million to \$55 million a year, replacing AT & T facilities for which the networks will be charged approximately \$70 million this year. The satellite system proposed in this

study would comprise three geosynchronous satellites each with the capacity to handle 12 colour television programmes together with television and radio audio and other communications facilities. Approximately 160 Earth stations, 30 capable both of sending and receiving and 130 for receiving only, would be required. An alternative ground microwave system, requiring about 600 towers to cover in excess of 18,000 "system miles", would operate in the common carrier frequencies at a nearly identical annual cost.

While the networks have not yet decided which, if either, system they would go forward with, Alan Cooper, NBC vice-president for planning, stated last week that while the present estimated costs were nearly identical, the satellite system would probably be preferable since its costs are more likely to decrease in the next few years as technology improves.

TelePrompter is another company that in recent months has been studying the possibility of launching its own satellite system. Over a year ago, when it was assumed that Comsat would be putting up the only domestic satellites, the National Cable Television Association proposed that CATV lease six programming channels from Comsat. Comsat was agreeable and felt that 36 Earth stations would be required to link up the six channels to most of the CATV areas. TelePrompter's president, Irving B. Kahn, argued that Comsat's prices were too high and the proposed system too limiting. He later offered to go in with the networks on their consortium when it became apparent that Comsat might not automatically be granted the domestic satellite market. The networks, who still fear the competition of CATV, were unresponsive, leading TelePrompter to commission Hughes Aircraft Company, a major shareholder in TelePrompter, to undertake a feasibility study. The study is investigating both the establishment of ground stations to coincide with CATV headends throughout the country and the launching and operating of an independent satellite. Although not yet complete, Hughes has already concluded that the ground stations themselves could be built for a great deal less than Comsat had originally estimated. TelePrompter's decision to launch its own satellites as well must obviously wait until completion of the study, but there seems little doubt that it will be another strong contender in the satellite sweepstakes.

But what about Comsat itself? At present the corporation is lying low and is seemingly calm about the burgeoning competition, but it is not sitting still. A spokesman for Comsat said last week that it would probably have a filing in within a few weeks. Its past proposals have been similar to that put forward in the Page study; the satellites themselves would be similar to the fourth generation Intelsat satellites being launched early next year with a capacity of 3,000 to 9,000 channels, while past Comsat proposals have contemplated over 100 ground stations.

Where the FCC will go once all these proposals have been filed is still unclear, but now that it has opened the door to companies other than Comsat, it seems unlikely that Comsat will be allowed to continue its exclusive control of the skies.

Introspection in Canada

THE fourth annual report of the Science Council of Canada should help to encourage the introspection and redefining of goals which are now going on in Canada's scientific community. In keeping with the many reports which have been published by the council during its four year lifetime, the report of its activities during the year ending March 31, 1970, is a closely argued plea for a more rational approach to the use of Canada's scientific and technological resources, and the report also points out some of the difficulties of living next to the United States.

The Science Council's fourth year was dominated by federal austerity, from which science and technology did not escape entirely unscathed. Dr O. M. Solandt, the council's chairman, points out, however, that the government was willing to listen to the arguments against cutting back on research expenditure, and made exceptions to its policy of financial restriction in some important areas, for example, the study of water resources and pollution.

The central theme of the report is that economic growth should not be the sole yardstick by which a country's progress should be judged, and that science and technology should not be slaves to a rapidly growing gross national product. Dr Solandt suggests that there is a crucial need for Canada to find reliable indicators other than GNP which will provide a better measure of the country's progress towards achieving national goals. Only when such a yardstick can be found can a set of criteria be drawn up for judging the possible benefits of a new technology. There is also, of course, the evergreen problem of defining national goals.

Part of the process of gearing science and technology to social improvement is the use of the currently fashionable technique of technology assessment, and

Dr Solandt argues that social scientists have a very great part to play in helping to weigh the costs and benefits of new technology. Only if a reliable mechanism for studying the likely long-term implications of a new project can be worked out will it be possible to guard against the misapplication of technology, he argues, and such a mechanism implies an effective teamwork between natural scientists, social scientists and engineers.

In Dr Solandt's view, living next door to the United States can be both a rewarding and a harrowing experience. He points out that the fortunes of Canada are becoming increasingly intertwined with those of the United States, and that Canada tends to import some of the United States' problems into its own cities and universities. There is still time, however, for Canada to take action before it finds itself confronted with some of the problems being faced south of the 49th parallel. One such problem is that the close links between science and the military-industrial complex have resulted in a real antagonism towards science, and this, Dr Solandt argues should be good reason for Canada not to develop an identifiable military-industrial complex of her own.

The chief effect of the proximity of the United States is the comparative ease with which American companies can penetrate Canadian markets and the ease with which Canada finds itself following American policies. "If Canada began to show more independence both in policy and action, as it is now doing in the matter of pollution in Arctic waters," Dr Solandt suggests, "far from causing strained relations between the two countries, in the long run it probably would increase US respect for Canada and Canadians."

As far as immediate problems are concerned, the report suggests that Canada's use of science and technology for development of the Arctic lacks coherence and organization, and that there is an obvious need to define goals for the area and then to mobilize scientific and technological resources to achieve them. There is also the problem of poverty among the Arctic population and the threat of pollution of the Arctic environment. In Canada's cities, the report suggests that there is an urgent need for special aspects of urban research, including transportation. Pollution also gets considerable attention in the report, but unfortunately the only real suggestion given is that more talent and money should be invested in the programme to clean up the rivers and the air.

Finally, Dr Solandt points out that the "major programmes" which were advocated in an earlier report of the Science Council have been very slow in developing. He suggests, however, that some of the blame for this can be laid at the Science Council's own door, because it has been slow to define suitable areas for such programmes, but the declining growth rate of public expenditure on science and technology has also played its part. One programme which has, however, made good progress is Canada's nuclear industry, although there still seems to be some difficulty in involving industry, especially in the design work.



O. M. Solandt, Chairman, Science Council of Canada.

NEWS AND VIEWS

The Universal Initiator

THE capacity to synthesize protein at 37° C in a dilute aqueous environment is virtually half the definition of life. It would therefore be naive to expect the basic pathways of protein synthesis to differ greatly between organisms that are as distantly related as rabbits and bacteria. Protein synthesis is too fundamental a property to have been left to the vagaries of parallel evolution. But on the face of things it would be equally naive not to expect numerous variations on the basic theme to have accumulated since, for example, bacteria and eukaryotes shared a common ancestor. What is so surprising is that these anticipated idiosyncrasies are proving so few and far between.

The story of how bacteria and eukaryotes make protein is proving to be virtually identical even down to the fine print (see pages 672 and 676 of this issue). The initiation of protein synthesis is a case in point. In bacteria the first amino-acid of every polypeptide chain is believed to be methionine, the amino group of which is blocked by formylation. Once the protein has been made, the formyl group is always cleaved and more often than not the methionine residue itself is also clipped from the polypeptide. Bacterial cells contain a unique species of methionine transfer RNA which is not only able to recognize initiation sequence in a messenger RNA but also allows the methionine residue to be formulated, so effecting chain initiation.

But to what extent does this scheme apply to eukaryotic cells? Is methionine the universal initiating amino-acid or is the blocked amino group the crucial characteristic of the initiator? In May, Smith, Marcker and Brown (*Nature*, **226**, 607 and 610; 1970) reported, as Caskey and others before them had done, that eukaryotic cells contain two species of methionine transfer RNA and although eukaryotes lack trans-formylases one of these two methionine tRNAs can be formylated by bacterial enzymes. As Smith and Marcker showed, this species acts as an initiator, incorporating methionine when synthetic messenger RNAs are translated in a cell free system from Ehrlich ascites cells. The other methionine tRNA incorporates methionine internally into the growing polypeptide chain. Moreover, Brown and Smith found that in a cell free system containing all the tRNAs only synthetic messengers with the methionine codon AUG near their beginning are translated.

Synthetic messengers are, however, one thing, natural messengers another. Real proof that methionine is the universal initiator depends on showing that it initiates synthesis of authentic proteins specified by natural messengers. Last year, Sherman and his colleagues reported indirect evidence that this is the case. They found that cytochrome *c* made in certain mutant strains of yeast begins with a methionine residue which is later removed, exposing the normal first amino-acid, threonine. Now Jackson and Hunter (see page 672) and Wigle and Dixon (see page 676) report respectively that rabbit alpha and beta globin chains and protamine in trout testis cells are initiated with methionine.

Jackson and Hunter used sodium fluoride to synchronize at the preinitiation stage a population of ribosomes in reticulocytes. They then removed the sodium fluoride and allowed globin synthesis to initiate but prevented extensive growth of the nascent polypeptides with sparsomycin. By isolating the short polypeptides and analysing their amino-acid composition they have been able to show that methionine initiated their synthesis. They are also able to follow the fate of the initiating methionine and conclude that it is cleaved from the growing globin chain once that is 15 to 20 residues long. Several other groups, whose work has not yet been published, have also by all accounts identified methionine as the initiator of globin synthesis.

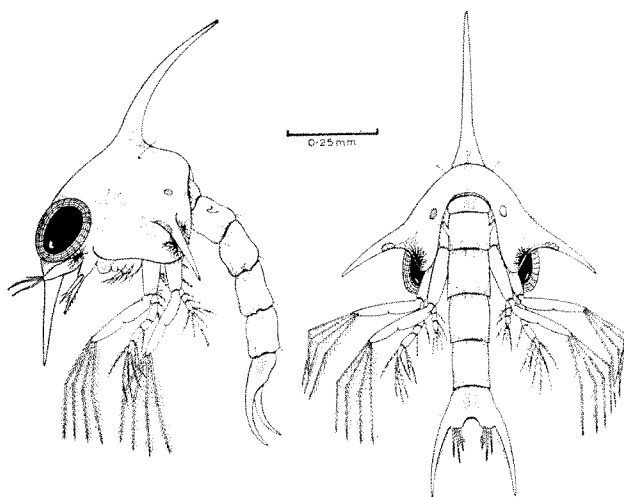
Wigle and Dixon's work comes as a great surprise. Trout protamines—arginine-rich basic proteins of low molecular weight—are apparently made on the small polysome comprising two ribosomes and protamine messenger. The advantage of the trout testis material is that it develops synchronously; the synthesis of the protamine occurs at the spermatid stage of development. By labelling a suspension of testis cells at this stage with methionine and extracting the methionine labelled protein, Wigle and Dixon have shown not only that methionine occurs at the N-terminal position in newly synthesized protamines but also that it is never formylated in nascent polypeptides. Moreover, extracts of testis cells contain an enzyme activity which can cleave the bonds between methionine and the second amino-acid, proline. The kinetics of labelling with methionine indicate that the initiating

residue is normally cleaved from the protamine, probably after its synthesis is completed.

Protamines are of course very unusual and specialized proteins. If methionine initiates their synthesis, as well as initiating synthesis of the more mundane globin, the odds must be that methionine is the universal initiator. The sole difference between initiation of protein synthesis in bacteria and eukaryotes boils down to the requirement in bacteria not just for methionine but for methionine with its amino group blocked by formylation. Perhaps the eukaryote ribosome with its increased complexity compared with the bacterial ribosome has during the course of evolution taken over the role of the formyl group and permitted this additional requirement of the initiating amino-acid to be dispensed with.

MARINE BIOLOGY

Young Crabs Described



This larva of the crab *Hemigrapsus crenulatus* is one of eight species described recently, together with a key for identification, by R. G. Wear in the *New Zealand Journal of Marine and Freshwater Research* (4, 3; 1970).

NUCLEIC ACIDS

Ends and Means

from our Molecular Biology Correspondent

IN 1967 Inman demonstrated that some parts of the DNA chain of λ phage undergo thermal melting at lower temperatures than others, and that these labile (presumably because AT-rich) regions could be observed and mapped by electron microscopy. The procedure was to equilibrate the DNA at a chosen temperature within the melting range, prop open the denatured segments with formaldehyde, cool the sample and spread it on a protein monolayer for electron microscopy. The melted regions occur predominantly in one half (arbitrarily designated the right half) of the chain, and form a kind of fingerprint of the DNA.

Since then denaturation maps have been observed for several DNA species, most recently that from a human adenovirus (Doerfler and Kleinschmidt, *J. Mol. Biol.*, **50**, 179; 1970). The molecule, which is

11 μ m long, shows, when 10–25 per cent melted, four well defined zones of denaturation. The value of this approach for a virus of this kind lies in the absence of any genetic characterization. The existence of a unique denaturation map, signifying an invariant distribution of AT-rich segments, is taken to reflect a unique, rather than a permuted, gene sequence.

It seemed from the outset, however, that so finely wrought a technique must find uses beyond the detection of gross variations in composition along the DNA, and this expectation has now been borne out by an accomplished study of the replication of λ DNA by Schnös and Inman (*ibid.*, **51**, 61; 1970). It was established by a group of Japanese workers that shortly after infection of *E. coli* by λ phage some molecules of circular λ DNA appeared, in which two points on the circle were linked by another loop of DNA. It was presumed that the presence of the branch points denoted, in accordance with the currently accepted scheme, that these molecules were in the process of replication. Schnös and Inman addressed themselves to the problem of determining the locus of the replication points. If the circles with loops indeed represent complexes of parent and offspring strands, then the sum of the long, presumed parental, segment between the branch points and the average of the two sides of the loop, or putative offspring segments, should be equal to that of the mature DNA, namely, 17.5 μ m. This turns out indeed to be so. Denaturation maps were then compiled, using alkali, rather than heat, as the denaturing agency, for this was found to give better results. The zones of denaturation, at low overall melting, in mature DNA occur at 8.3–10.6 μ m at 12.7–14.0 μ m and at 16.2–17.1 μ m from the left hand end, and in addition a small zone appears at the left hand terminus. The circular and looped circular species give precisely similar maps as the linear DNA, and the characteristic zones can then be used to index the branch points along the DNA. The loops of offspring DNA could be seen, when they were long enough, to possess identical melting maps to the parental material.

The looped circles that were found corresponded to varying degrees of replication, up to 90 per cent, and fell into three distinct populations: in one the right hand branch point was fixed, irrespective of how far round the circle the other end reached, in another the left hand end was fixed, and in the third and predominant type, neither end was fixed. Where there was a fixed branch point, this was always rooted some 14.3 μ m from the left end (referred to linear DNA), and indeed a plot of the position of the migrating end against the extent of replication was linear, extrapolating to the origin at 14.3 μ m. Similarly, when both branches moved outwards around the circle they did so from this same initiation point, though, as was apparent from the spread of branch positions, not always at exactly the same rate. Evidently then replication occurs most commonly, though not always, on both strands at once, starting from an initiation point 14.3 μ m from the left end. This is known to correspond to the position of the so-called P-gene, and is in a highly GC-rich area. The results seem to dismiss previously propagated notions of replication in one direction only.

It may be noted that after slight thermal denaturation of λ DNA, and rapid cooling, in the absence of formaldehyde (Fukey, Wada and Tomizawa, *ibid.*, 255),

melting is observed only at the extreme right hand end. Evidently, in these circumstances, any internal regions which have melted immediately reanneal when cooled.

SEA FLOOR SPREADING

Non-Axial Symmetries

from our Geomagnetism Correspondent

THE basic premise of the sea floor spreading hypothesis is simply that mantle material rises at mid-oceanic ridges, becomes magnetized in the direction of the ambient geomagnetic field, and moves away laterally and at right angles to the ridge axis to form new ocean floor. There are a few situations in which a more complicated model is required; but, by and large, spreading from a single ridge seems to fit the observations very well.

But into this conceptually simple works, Havemann (*Earth Planet. Sci. Lett.*, **8**, 451; 1970) has thrown a spanner. How big and strong the spanner proves to be only time will tell; but it will no doubt be examined carefully by those who feel that nature cannot be, *a priori*, as simple as sea floor spreading seems to be. For Havemann has noticed that the magnetic anomalies in the South Pacific, the North Pacific and the South Atlantic are symmetrical about the line separating anomalies 25 and 26 as well as about the ridge system. This symmetry seems to lie between anomalies 19 and 32; that is to say, anomalies 25 to 19 are the mirror image of anomalies 26 to 32.

Havemann's explanation of this unexpected discovery is that there may once have been an active ridge between the present anomalies 25 and 26, which is now extinct. In this case the whole sequence of anomalies from 1 to 32 would not have originated by spreading in the same direction. By the same token, the anomalies would not then be in age sequence— anomalies 25 back to 19 would be in inverse sequence. In principle, of course, this suggestion is testable by direct sampling and dating; and will no doubt be tested when enough samples have been collected in the relevant regions.

Meanwhile, is there any evidence that a hiatus in spreading followed by the appearance of a new spreading ridge elsewhere is either possible or likely? There may be. Havemann notes, for example, that the Darwin Rise has subsided and its activity apparently transferred to the East Pacific Rise. A similar displacement may also have taken place, although on a smaller scale, from the Juan de Fuca Ridge which lacks any recent seismic and volcanic activity. Bostrom (*Pacific Geol.*, **1**, 1; 1968) has already suggested that there may be a sub-continental ridge along the coasts of Oregon, Washington and British Columbia.

According to Havemann, the symmetry across anomalies 25 and 26 occurs on one side of the mid-oceanic ridges, but cannot be seen on the other side because the data are unavailable. If it turns out that the symmetry exists separately on both sides, there would, of course, be a problem with the extinct ridge hypothesis. But Havemann is hopeful that such a doublet could be explained satisfactorily. He points out that the East Pacific Rise has a more rift-like topography and higher heat flow on its flanks than on its central line. It may be that an active ridge gradually separates into two ridges which spread apart. Subsequently they may then become extinct followed

by a period of rest and then a return of active spreading to the original central ridge.

Or is it all just an unhappy coincidence?

CRAB NEBULA

Full of Surprises

from a Correspondent

THE Crab Nebula, the remnant of the "guest star" seen by the Chinese in 1054 AD, has had a history of surprises. Hubble first realized the connexion between the ancient supernova and the present nebula when the large expansion motions of about 1,000 km/s⁻¹ were measured. It was the first radio source to be identified by Bolton and Stanley in 1949. Then came evidence of continuing optical activity with light fronts moving outwards from its centre. In 1963 Friedman *et al.* found X-ray emission from the Crab Nebula. The surprises culminated in 1968 in the discovery, by the group at Arecibo, of a pulsar at the centre of the nebula. This identification of neutron matter, the most condensed state of matter in the universe, has excited new interest in the Crab Nebula and its associated pulsar. In the light of this interest the International Astronomical Union sponsored a symposium at Jodrell Bank from August 5 to 7, to make an intensive study of the relationship between the nebula and the pulsar, and to ask how representative they were of other pulsars and supernova remnants.

Dr F. D. Drake (Cornell), reviewing the radio properties of the Crab pulsar, emphasized the variability in the radio pulse in contrast to the optical pulse, where no changes down to a level of several per cent have been observed for about a year. Because the radio pulse can vary by more than a factor of 100, it is possible to investigate the detailed structure of individual strong pulses as well as the mean properties of the pulsar. Drake summarized the data from several observatories, which point to the identification of the Crab pulsar with the compact low frequency radio source at the centre of the nebula, discovered at Cambridge in 1964. The short rotation period (33.1 ms) precluded its identification with conventional optical techniques and, as several speakers pointed out, the pulsed characteristic is largely smeared out at radio frequencies below about 80 MHz, because of multipath propagation effects in the interstellar medium, which broaden the pulse as the fourth power of the wavelength.

In spite of the intensity variability it is possible to obtain extremely accurate radio timing data to complement the optical data with a precision of 1 μ s within a year. (General relativistic effects are 1,500 μ s due to the Earth's motion.) The most intriguing event shown up by the timing observations was the jump in period on September 26, 1969, which corresponded to a decrease in radius of 10 μ m. The gravitational energy released from this minute adjustment in radius was enormous, enough to supply the kinetic energy of one of the wisps found by Scargle to move out from the central star, as reported by Dr V. Trimble (Institute of Theoretical Astronomy, Cambridge).

The interpretation of these timing data was a controversial topic. Dr D. T. Wilkinson (Princeton) interpreted his results as indicating the presence of one or possibly two planetary companions of the pulsar, and he predicted a similar jump to that observed on September 26, 1969, for April this year. But people

who had timing data for this period were not prepared to admit the presence of a jump of similar magnitude, and were inclined to seek an explanation in the processes occurring nearer the surface of the neutron star.

Dr L. Woltjer (Columbia) noted the well established fact that the energy released from the continuing slowdown of the pulsar is comparable with the energy radiated from the nebula, and he emphasized the high efficiency (20–100 per cent) of accelerating electrons and protons to relativistic energies. Although the precise mechanism is not agreed, pulsars can efficiently provide sources of highly relativistic particles in the Galaxy.

Are the Crab Nebula and its associated pulsar unique? Dr D. K. Milne (Radiophysics Division, CSIRO, Sydney) concluded from an extensive survey of about 100 radio supernova remnants that there may be several with the same centrally filled structure as the Crab. These are, however, more evolved, and clearly there is no other source of the same age as the Crab Nebula in the Galaxy. A similar conclusion was reached for the Crab pulsar—none of the same age have been detected, but several have similar pulse shape and polarization characteristics.

Many problems remain. What, for example, is the role of general relativity in the high densities of neutron stars? Why do all the 55 pulsars known have a pulse width close to 3 per cent of their period? Professor F. G. Smith (Jodrell Bank) reminded delegates that although many pulse characteristics could be conveniently explained in terms of the generally accepted oblique rotating magnetic field model, there were difficulties in explaining the high degree of circular polarization at the edge of the pulses. He suggested that this is some maser amplification process operative at radio wavelengths.

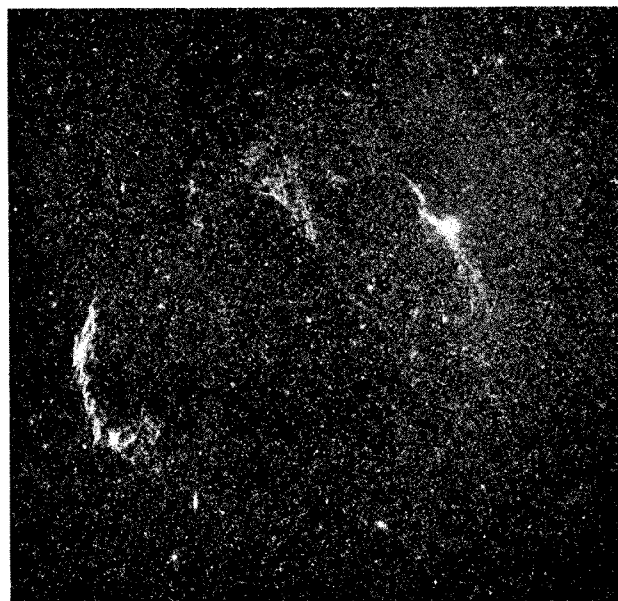
SUPERNOVAE

X-rays from Cygnus Loop

from our Observatories Correspondent

THE detection of soft X-rays from a source (Cygnus X-5) which is thought to be the Cygnus Loop has been reported recently in *Astrophysical Journal Letters* (161, L45; 1970). The discovery was made by a team from the Lawrence Radiation Laboratory of the University of California, R. Grader, R. Hill, and J. Stoering, who used rocket-borne equipment.

The Cygnus Loop is the classical example of an old supernova remnant. It is composed of very fine filaments of emitting nebulosity which comprise two large diametrically opposed segments of a circle, about a degree across in the sky. (Portions of these filamentary structures, which by and large are tangential to the circle, are frequently used as illustrations in popular books on astronomy under such beguiling names as the "veil nebula" or the "network nebula".) In 1946 J. H. Oort suggested that the filaments form an expanding shell which resulted from the explosion of a supernova some 50,000 years ago and that the emitting gas is excited by collision with the ambient interstellar medium. E. Hubble had earlier shown that the fine filaments are indeed moving outwards at a measurable rate, about 3 seconds of arc per century. In 1958 R. Minkowski showed from spectroscopic measurements that the radial expansion velocity of the shell is about 100 km s^{-1} . The luminous filaments seem to be shock fronts moving at about this speed into the



Cygnus Loop

undisturbed interstellar gas. The gas immediately behind a shock front moving at 100 km s^{-1} would be ionized and heated to a temperature of a few hundred thousand degrees. Minkowski's use of both the radial and transverse components of the motion enabled him to estimate that the shell is at a distance of about 770 parsecs and that its diameter is about 40 parsecs. A sphere of this diameter in the galactic disk would be expected to contain about 2,000 solar masses of interstellar neutral hydrogen which in this case has been swept up by the shock front created in the initial supernova explosion.

The LRL group detected X-rays from the Cygnus Loop in the energy range 0.1 to 2 keV with a peak at 0.2 keV. This fall in emission below 0.2 keV can plausibly be attributed to absorption by interstellar gas. The experimenters are not certain of the origin of the emission. The observed X-ray spectrum above 0.2 keV can be fitted either by a power law of the kind produced by synchrotron radiation or by an exponential which would be produced by thermal bremsstrahlung from a hot gas at about 4 million K. A thermal origin seems to be the more likely hypothesis, although the required temperature seems a little high in view of the known expansion velocity of the shell. Nonthermal radio emission is observed from the Cygnus Loop. According to H. van der Laan it is synchrotron radiation produced by galactic cosmic ray electrons as they traverse the compressed interstellar magnetic field behind the shock front.

Whichever theory turns out to be right, it seems that X-ray observations will give important data on the evolution of supernova remnants from relatively young objects such as the Crab Nebula to objects as old as the Cygnus Loop.

ATOMIC PHYSICS

More Uses for Lasers

from a Correspondent

ADVANCES in experimental techniques reported at the international conference on atomic physics, held in

Oxford from July 21 to 24, included improvements in methods of trapping free ions for sufficiently long times (milliseconds) to permit very narrow cyclotron resonance linewidths to be obtained. These developments, which should lead to considerable improvements in precision of magnetic moment ratio determination, and collision frequency measurements, were reported by Professor M. Bloom (University of British Columbia) and Professor A. Rich (University of Michigan). Observations of weak absorption lines in the solar spectrum, and the advances in spectrophotometric techniques which made such observations possible were described by Professor D. E. Blackwell (University of Oxford). Improvements in spectroscopic techniques, including such refinements as interferometric measurement of reflector displacement using super-radiant laser sources in Fourier transform spectroscopy, were further detailed by Dr P. Boucharcine (Orsay).

A new technique for producing high densities of spin polarized atoms, ions and electrons was announced by Dr L. D. Scheerer (Texas Instruments Inc.). The helium triplet metastables in a flowing helium afterglow acquire a net polarization of about 30 per cent by absorption of circularly polarized light from lamps irradiating the afterglow stream. As a result of Penning reactions and charge transfer collisions, the polarization can be transferred to excited and ground state ions of gases or vapours titrated into the gas stream. Radiofrequency resonance measurements have thus been made on various ions, many of which were previously considered inaccessible to optical pumping.

Professor S. Bashkin (University of Arizona) described recent results of oscillator strength measurements and assignments of spectral lines from multiple ionized species observed for the first time in laboratory sources, obtained using the beam-foil technique.

Professor A. Javan (Massachusetts Institute of Technology), describing his work on beating laser frequencies in the infrared with harmonics of electronically generated microwave signals, announced that all the individual steps in the chain of procedures which would allow determination of the frequency of a visible gas laser in terms of the caesium clock standard had been demonstrated. Vital to the establishment of a bridge between microwaves and infrared was the development of a mixing diode which uses a hair-like antenna wire of a few microns diameter, tapering at the rectifying contact to a tip of radius 500 Å. Dr H. Walther (Joint Institute for Laboratory Astrophysics, Boulder, Colorado) announced the development of a dye laser with a narrow frequency band output. The possibility of concentrating the laser's normally broad band output into a frequency range no wider than a few hundred MHz, while retaining the ability to tune the output across most of the visible spectrum, was realized by combining a grating end reflector with a Lyot filter inside the cavity. Comparisons of evidence available from studies of isotope effects in optical spectroscopy with predictions of nuclear models and the theory of isotope effects in muonic X-ray spectra were reviewed by Drs D. N. Stacey (Oxford) and R. C. Barrett (Surrey).

Professor E. W. Otten (Heidelberg) described work alignment of extremely small (less than 10^6 atoms) samples of radioactive nuclei, produced by bombardment of rare-gas atoms and polarized in spin-exchange collisions with optically pumped rubidium. Asymmetry

of the beta-decay provided the diagnostic tool for detection of radiofrequency resonance.

In the final session Professor J. G. King (MIT) described an interesting and novel technique for detecting the quantized vortex structure of superfluid helium through the interaction with an atomic beam.

CONTINENTAL DRIFT

When did Gondwanaland Split?

from our Geomagnetism Correspondent

As far as the continents around the Atlantic Ocean are concerned, one of the most authoritative and widely accepted pre-continental drift configurations is the purely geometrical fit based on a least squares analysis of continental margins at the 500-fathom contour (Bullard *et al.*, *Phil. Trans. Roy. Soc., A*, **258**, 41; 1965). Recently two attempts have been made to test it using palaeomagnetic evidence. The first utilized all relevant Permian and Carboniferous pole positions available to 1964, irrespective of whether or not the rocks concerned had been magnetically cleaned. The results of this test were inconclusive, probably because of the inclusion of the less reliable data. A second attempt was made in 1968, using only data from rocks which had been adequately cleaned. And again the results were inconclusive, simply because there were too few reliable data.

Larson and LaFountain (*Earth Planet. Sci. Lett.*, **8**, 341; 1970) have made a third attempt to test the Bullard fit palaeomagnetically because they feel that there is now enough good data available to make it worthwhile. Their conclusion, based on an analysis of Permian and Carboniferous pole positions, is that the Bullard fit is valid within the limits of the technique. But, what is perhaps more important, by extending the polar analysis to the Triassic and Cretaceous, Larson and LaFountain have determined, with greater precision than before, the time at which the continents began to drift apart.

The technique they have adopted is to hold North America stationary and rotate the other continents into the relative positions indicated by the Bullard fit. When this is done, Carboniferous poles from Eurasia and North America, which were widely separated before the rotation, move to near coincidence. Permian poles from North America, Eurasia and Africa show the same behaviour. From this it is a fair inference that during the Carboniferous and Permian, Europe, North America and Africa were contiguous or almost so. The situation with regard to the other major continent, South America, is less certain; but in so far as the few available poles move towards the common pole when rotated, the same conclusion can justifiably be drawn.

In the Triassic the polar coincidences after rotation are not quite so good, which would seem to indicate that this was when the continents began to split. This is confirmed by the behaviour of the Cretaceous poles. Rotation of the African poles to the Bullard fit produces first a convergence and then an increased scatter. This would be expected if the rocks concerned had been magnetized since drift began. Furthermore, the closest coincidence of North American and African Cretaceous poles occurs about halfway

along the rotation path. This can only mean that by the Cretaceous (80–100 million years) the South Atlantic had already opened by about 50 per cent, having begun to open during the Triassic about 200 million years ago. Rotation of the Eurasian poles, on the other hand, produced divergence right from the start. By about 100 million years ago, therefore, the North Atlantic (above 40° N) must have been about as wide as it is now.

It must be said, however, that the time of break-up of the continents (200 million years) determined in this way does not agree with that deduced from Atlantic sea floor spreading (less than 100 million years). Which, then, if either, is correct? The discrepancy could arise from incorrect palaeomagnetic pole positions, wrong sea floor spreading rates, or possibly a situation in which the Atlantic sea floor is spreading beneath the continental edges. There is no evidence for the last of these—there are, for example, no earthquake zones as there are near the descending Pacific Ocean floor—and it is impossible to evaluate properly the possibility of incorrect pole positions. But, as Larson and LaFountain point out, the sea floor spreading determination is the most likely to be incorrect. Times of continental break-up determined from sea floor spreading are based on linear extrapolations of spreading rates during the past five million years. Only a brave man or a fool would deny the strong possibility of error in an extrapolation from 5 million to 100 or 200 million years. The best estimate for the time of break-up of Gondwanaland is thus 200 million years ago.

MATERIALS

Explosive Preparations

from a Correspondent

DIVERSE aspects of materials forming processes were the theme of a conference held at Swansea by the Materials Science Club on July 23 and 24. Setting the scene, Dr B. Lengyel (Imperial College, London) discussed engineering limitations to forming processes, such as the vessel size in hydrostatic extrusion. For high speed metal forming processes, it is important to relate temperature, strain, strain rate and pressure effects, for there are certain advantages in operating at room temperature and high strain rate. Temperature effects introduced by converting plastic work to heat must be considered, however, particularly with regard to heating of the die and interface with a subsequent change in the interfacial lubrication conditions. Pressure increases the strain at fracture and has great potential for extrusion of brittle materials. Furthermore, because voids can be induced to heal, effects can be achieved similar to those obtained in hot working.

Discussing the technique of high velocity forming (HVF), Dr A. J. Organ (University of Birmingham) identified three process categories: stroke restricted, power restricted, and work restricted. In pneumatic mechanical forming practice, a high gas pressure is applied to a ram to give it a high velocity (say between 10 and 60 feet per second), and on impact the kinetic energy is equivalent to the plastic deformation of the workpiece. When HVF was applied to cropping, it

was pointed out, there was usually no wastage of materials and the process was cheaper than sawing.

In explosive forming, much greater velocities are used (10^3 – 10^4 feet per second), and because of the nature of the operation it is usually done out of doors. Mr E. Amini described how this technique has been refined at the University of Birmingham using fairly simple equipment to enable 2 foot diameter disks to be explosively formed indoors using moderately low explosive charges.

Two major recent developments have revolutionized the use and fabrication of glass. Mr H. Charnock (Pilkington Ltd), who reviewed the float glass process, emphasized particularly the importance of familiar surface chemistry equations for predicting the glass plate thickness, and suggested engineering solutions to the problem of producing glass of various thicknesses. In the second case, although the uses for fibre optics are still developing, Mr C. I. Western (Rank Kershaw) said that significant advances have been made in the manufacturing processes. There are clear distinctions in the techniques for producing randomly oriented fibre bundles for light transmission and those for producing coherent bundles of two million individual fibres for picture transmission.

Reviewing recent advances in the fabrication of ceramics, Dr D. I. Matkin (Atomic Energy Research Establishment, Harwell) discussed the developing technique of incorporating a certain amount of organic plastic into the ceramic powder. The result may be formed by extrusion or injection moulding and the plastic subsequently burnt out in part of the firing process. If silicon resin is used, the technique can be adjusted so that the final product contains silica. Continuous hot pressing represents a break-through in ceramic fabrications. If two plungers are used, and the top one can be removed to introduce more powder after each compaction, together with a specially designed die, the material pressed eventually becomes the bottom plunger enabling long ceramic rods of simple shape to be formed.

To form engineering components successfully in plastic requires not only a simple technique to keep costs at a minimum, if the advantage of a low-priced material is not to be lost, but also the expertise of the tool designer. Dr M. Abrahams (GKN Ltd) described a technique for hydraulic pressing of plastic components from injection moulded billets. In the final product the tensile properties are non-uniform in the radial direction; the technique is considered to be complementary to the injection moulding of components, but whether or not the change in properties produced is beneficial in the long term remains to be seen. Mr I. T. Barrie (ICI Ltd, Plastics Division) discussed how conventional melt processing of plastics is being modified for a more discerning market. For example, the production of the integral plastic hinge, as an accelerator pedal, is an interesting example of the way the melt flow behaviour into the mould requires careful study to optimize the conditions to give satisfactory properties. Again, injection moulding processes must be better understood if larger components are to be formed without the need for scaling the equipment consistent with present operational experience. A good example is the production of car bonnets, which has been achieved using a piston approach to the mould design to produce these large components at moderately low operating pressures.

Phytochrome and Photomorphogenesis in Plants

by

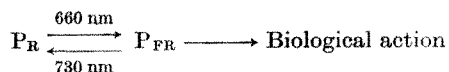
HARRY SMITH

Botany Department,
University of Manchester

Much of the information available about the behaviour of phytochrome in plants can be brought together into a model involving association of the pigment with membranes.

MANY aspects of morphogenesis in higher plants are regulated by light. Typical examples include the photo-periodic control of flowering and bud dormancy, the regulation of leaf and stem expansion, the stimulation or inhibition of seed germination and the biosynthesis of various pigments (reviews in ref. 1). During the two decades before 1960, intensive investigations of the action spectra and energy requirements of various photoresponses, led principally by Drs H. A. Borthwick and S. B. Hendricks at the US Department of Agriculture Research Station at Beltsville, culminated in an elegant and intellectually satisfying unification.

It had been found that many photomorphogenetic phenomena had very low energy requirements and were reversibly potentiated and negated by short periods of red light ($\lambda_{\text{max}} = 660 \text{ nm}$) and far-red light ($\lambda_{\text{max}} = 730 \text{ nm}$) respectively, the nature of the ultimate response being determined by the final irradiation treatment². On the basis of this evidence, the Beltsville group predicted that the photoreceptor (P) existed in two photoconvertible forms: P_R , absorbing maximally at 660 nm, and P_{FR} , absorbing at 730 nm, and that the form produced by red irradiation was responsible for the subsequent developmental events. Thus:



In 1959, this prediction was decisively borne out by the direct demonstration of the expected absorbancy changes in living tissues, and by the successful extraction of the photoreversible pigment as a chromophoric protein³. At this stage, the role of phytochrome, as the pigment was named, seemed reasonably clear and uncomplicated; it only remained to discover the nature of the initial primary reaction catalysed by the presumed enzyme, P_{FR} . This concept of phytochrome action was truly germinal, and since that time investigations into the nature of phytochrome action have increased enormously in quantity, ingenuity and complexity. Unfortunately, although this work has substantially increased knowledge of phytochrome, it has also, perhaps inevitably, considerably confused the overall picture. Hypotheses of the action of phytochrome in specific developmental phenomena have proliferated and the unity of the early ideas has been largely lost. It is my purpose here to attempt to draw together these divergent trends and to clarify as far as possible the general picture for the non-specialist reader.

Phytochrome a Protein

Phytochrome has been isolated and purified by several groups and shown to be a protein, probably existing in monomeric and polymeric forms, the monomer with a molecular weight of about 42,000⁴. The chromophore is a linear tetrapyrrole of the bilatriene configuration⁵, but the complete structures of the P_R and P_{FR} forms are not yet fully worked out. There is evidence for the existence of multiple chromophore forms in at least one plant

species⁶. Flash spectroscopy investigations of the *in vitro* photoconversions of P_R to P_{FR} and *vice versa* have shown the existence of several intermediates and partial reactions with differing rate constants⁷. Evidence has also been adduced for the existence of "leuco" or colourless forms of both P_R and P_{FR} ⁸ and of long lived intermediates⁹. Thus the photoconversions are chemically quite complex. They are further complicated by associated changes in the tertiary structure of the protein moiety of the molecule¹⁰. The binding of the chromophore to the protein is very strong, and isolated chromophore material does not have the capacity for photoreversible conversions⁶. The conversions, therefore, are properties of the whole, intact molecule. Non-photochemical conversions also occur in isolated phytochrome, in particular a relatively slow reversion of P_{FR} to P_R , and a conversion of P_{FR} to an unknown, photochemically inactive, derivative¹¹.

The absorption spectra of the two forms (Fig. 1) have overlapping absorption bands which render total photochemical conversion impossible. Thus 660 nm light produces an equilibrium of approximately 80 per cent P_{FR} and 20 per cent P_R , while the proportion of P_{FR} present under 730 nm irradiation is usually less than 3 per cent of total P ¹². Similar considerations apply to photochemical conversions *in vivo*. Non-photochemical conversions also occur *in vivo* but seem to be kinetically different from those of purified phytochrome¹³. The dark reversion of P_{FR} to P_R , originally thought to be widespread and to be implicated in the time-measuring mechanism in photoperiodism, is now considered to be restricted to only certain dicotyledonous tissues, including etiolated pea and bean shoots¹³, and cauliflower head tissue¹². Thermochemical transformation of P_{FR} to a photochemically inactive form (a process known as "decay" or "destruction") is widespread *in vivo* but, in contrast to the similar *in vitro* conversion, is dependent on metabolic activity¹⁴. The decay process is restricted to P_{FR} and is in most cases a first order reaction, the total loss of P being dependent on the equilibrium proportions of P_R and P_{FR} in the particular conditions. Thus decay is rapid under

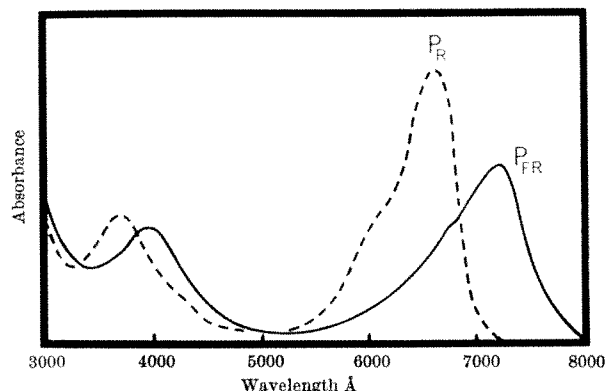


Fig. 1. The *in vitro* absorption spectra of the P_R and P_{FR} forms of phytochrome. (By permission of Dr K. M. Hartmann, ref. 37.)

red irradiation, which maintains a high proportion of P_{FR} , and is slow under far-red irradiation, which maintains a low proportion of P_{FR} . In some etiolated tissues, an apparent resynthesis of P_R can be observed in darkness, occurring only when the concentration of total P has been reduced below a critical level¹⁵. It is important to remember that all *in vivo* determinations of phytochrome are dependent on the changes in absorption at 660 and 730 nm as measured, usually, by a dual-wavelength difference photometer specifically designed for dense, light scattering samples¹⁶. Thus, "losses" and "gains" of phytochrome refer to the disappearance or appearance of spectral photoreversibility and do not necessarily involve major changes in the phytochrome molecule.

Considerable effort has been expended on attempts to determine the location of phytochrome within the plant as a whole, and within the cell. It is now generally agreed that phytochrome is concentrated in the meristematic and elongating regions of etiolated seedlings, whether it is measured by *in vivo* photometry or by extraction¹⁷. Seedlings grown in the light, however, contain such low concentrations of phytochrome that it is not possible to determine accurately the regions of highest concentration. The problem of cellular location is even more obtuse, for the solubility of the pigment is markedly dependent on pH. Thus, when alkaline buffers are used, phytochrome appears predominantly in the final supernatant on differential centrifugation, while slightly acidic buffers may cause its spurious precipitation onto organelles. Evidence for the possible localization of phytochrome in the nucleus or nuclear membrane of etiolated *Avena* cells comes from Galston's¹⁸ observation of photoreversible spectral changes using a microspectrophotometer. The observed spectral changes are unexpectedly large for such a small path length, however.

The most convincing evidence of the cellular location, however, is the demonstration by Haupt¹⁹ of the dichroic orientation of the pigment molecules in the plasmalemma of *Mougeotia*, a filamentous alga with a flat, plate-like chloroplast which rotates within the cell in response to the photoactivation of phytochrome. Haupt²⁰ has also shown, using microbeams of polarized red and far-red light, that the axis of orientation of the chromophores is parallel to the plasmalemma surface in the P_R form, but is changed to an orientation at 90° to the surface of the plasmalemma when photoconverted to P_{FR} . The localization of phytochrome in the plasmalemma of *Mougeotia* does not, of course, prove that it is similarly located in the other organisms, but it seems reasonable at present to conclude that phytochrome is usually a constituent of certain cellular membranes. This conclusion is supported by the physiological evidence described later.

Several workers have used the *in vivo* photometric method in attempts to determine the extent of correlation between the proportion of phytochrome converted by a particular light treatment and the final morphogenetic responses. In most etiolated plants, the correlation is very good, the final response being proportional to the logarithm of the percentage P_{FR} set up by a single irradiation^{21,22}. In plants grown in the light, however, it is not possible to carry out such experiments, and even in some etiolated tissues the correlations are non-existent. Two of the most striking examples have been dignified by the name "paradox". In the *Zea mays* paradox²³, light energies so low that they do not establish photometrically detectable amounts of P_{FR} will saturate the decrease in phototropic sensitivity which is mediated by red light.

Even more mystifying is the fact that this effect can be reversed by energies of far-red irradiation that are known to establish a higher proportion of P_{FR} than the low energies of red light used. In the *Pisum* paradox²¹, epicotyl segments, cut from etiolated seedlings the growth of which has been inhibited by brief irradiation with red light 8 h previously, are stimulated in their growth by far-red light even though photometric assays show that all P_{FR} established by the prior red light has disappeared (through dark reversion and decay). The phenomenon of far-red reversibility in the absence of detectable P_{FR} is widespread and, together with the *Zea* paradox, has led to the postulation of "bulk" and "active" forms of phytochrome, the bulk form being photometrically detectable in etiolated tissues but, at least in some cases, physiologically inactive. Another difficulty for the simple correlation of the ratio of P_{FR} to P with physiological effect comes from the report of a threshold of P_{FR} for repression of lipoxygenase synthesis in mustard seedlings²⁴. The absence of a graded effect suggests that P_{FR} functions through an all or nothing control mechanism of high precision.

Hormones and Membranes

Early work on the energy-response relationships of several photoresponses suggested that P_{FR} was the active form and that its action was relatively slow and cumulative. It now seems likely that the primary action of phytochrome can occur very soon after photoconversion, although the intervening processes between this primary reaction and the developmental events may take hours or days. The most striking example of rapid action is the phytochrome regulation of the closing of the leaflets of *Mimosa pudica* and related legumes, in which the photoconversion and the response are separated by less than 5 min²⁵. Other rapid responses are also known. The germination of 'Grand Rapids' lettuce seeds can be promoted by red light or by gibberellin. In the presence of sub-threshold levels of gibberellin, the red light stimulation of germination is not reversible by far-red light, even when given immediately after the red irradiation²⁶. This suggests that a product of P_{FR} action interacts immediately with gibberellin. Rapid action of phytochrome has also been found in the unrolling of cereal leaves²⁷ and in the photoperiodic control of flowering²⁸. It is thus clear that hypotheses of the mechanism of action of phytochrome must take into account the rapid action evident in these responses. In 1967 Hendricks and Borthwick²⁹ made yet another significant contribution to the subject by postulating that phytochrome is a controlling factor in membrane permeability, and that the rapid and slow responses both stem from this primary membrane effect. The basis of this hypothesis is the regulation of *Mimosa* pinnule movements which are thought to be caused by massive changes in osmotic potential in the pulvinal cells, brought about by changes in membrane permeability. A more dramatic response which is also thought to involve changes in membrane properties is the red/far-red photoreversible adherence of barley root tips to a glass surface³⁰. A rapid change in the electrostatic properties of the root surface is clearly involved here and, in fact, red/far-red changes in electrical potential have been reported³¹. Thus, although it cannot yet be regarded as conclusive, evidence is building up for the localization of phytochrome in certain critical membranes and for its having a function in the regulation of membrane properties.

Phytochrome and Gene Expression

The membrane hypothesis has tended to supersede the slightly older hypothesis, proposed by Professor Hans Mohr of the University of Freiburg, that phytochrome interacts specifically with the genome to regulate gene expression and thus to determine long term morphogenetic events through the induction and repression of specific enzymes³². That such a hypothesis cannot account for the rapid effects of gibberellin on lettuce seed germination which I have described makes it seem unlikely that P_{FR} interacts directly with the genome. On the other hand, many long term phenomena controlled by phytochrome can be inhibited by actinomycin D and inhibitors of protein synthesis in a manner which supports the involvement of enzyme synthesis³³. Furthermore, direct observation of phytochrome has been observed, a particularly good example being phenylalanine ammonia-lyase, a critical regulatory enzyme in flavonoid biosynthesis³⁴. The two hypotheses, of course, are not mutually exclusive, and may operate independently or even sequentially. For example, the primary change in membrane properties may lead to the transfer of an important metabolite, perhaps a hormone, from one membrane-bound compartment to another in which it can interact with other substances (for example, gibberellins) and from which it can go on to regulate, in some way, enzyme synthesis and the ultimate morphogenetic phenomena.

At this stage it must be pointed out that most investigations of the role of phytochrome have been concerned solely with plants grown in the dark. Photomorphogenetic phenomena, however, are by no means restricted to etiolated plants, and it is of importance to consider phytochrome in the context of the rather more "natural" light-grown state. As I have said, phytochrome has been extracted from some light-grown plants, although many plants, even some of those exhibiting definite red/far-red photoreversibility of flowering, yielded no detectable phytochrome³⁵. This is presumably because of the loss of spectral photoreversibility of phytochrome through the process of decay, and poses two complementary problems for the physiologist. On the one hand, what is the function of the relatively very large amounts of phytochrome in dark-grown tissues; and on the other hand, how can photomorphogenetic phenomena be regulated in a typical phytochrome fashion in light-grown plants that contain no detectable phytochrome?

Many photomorphogenetic phenomena are known which require prolonged irradiation with sources of relatively high energy. Such processes, which have been grouped under the collective term "the high energy reaction", typically do not show red/far-red photoreversibility and have action maxima above 700 nm, although some have other action maxima in the blue regions of the spectrum³⁶. Hartmann³⁷ has constructed a hypothesis which satisfactorily accounts for the >700 nm action maxima on the basis of phytochrome (the activity in the blue region is thought to be due to other photoreceptors; for example, carotenoids or flavins). In Hartmann's view, the far-red wavelengths establish an equilibrium between P_R and P_{FR} in which P_{FR} is in low concentration; thus the rate of disappearance of total P due to decay is low. Under continuous illumination with wavelengths which maintain a high proportion of P in the P_{FR} form (for example, between 600 and 680 nm) the rate of loss of total P is much greater. Maximum developmental effect is obtained in conditions in which low concentrations of P_{FR} are

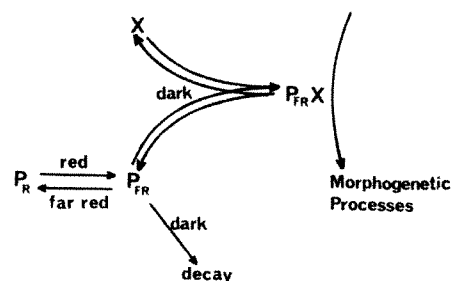


Fig. 2. Hartmann's hypothesis for the explanation of the high energy reaction on the basis of phytochrome³⁷.

maintained for maximum periods, that is, under far-red illumination. The hypothesis, which is represented in Fig. 2, has been tested successfully in several plant tissues utilizing mixed sources of red and far-red irradiation. In extension growth of lettuce hypocotyl³⁸, for example, maximum inhibition is obtained under continuous irradiation with a sharp maximum at 716 nm, under which approximately 3 per cent of total P is present as P_{FR} . If lettuce hypocotyls are irradiated with a standard energy of 768 nm and simultaneously with varying energies of 658 nm (neither of which wavelengths have any effect independently), a level of 658 nm energy is reached which gives strong inhibition. Above this level, the inhibitory effect is gradually diminished. Calculation of P_{FR} levels shows that inhibition is obtained with mixtures of 658 and 768 nm energies which maintain approximately 3 per cent of total P as P_{FR} . Other similar tests also support the Hartmann hypothesis as far as the involvement of phytochrome is concerned, although it is difficult to explain the observed intensity dependence of many high energy reactions on the basis of this hypothesis. Furthermore, the hypothesis has been criticized³⁹ for its assumption that all, or at least a constant proportion, of the phytochrome present in a tissue is active and that decay as seen in etiolated tissues is of physiological importance. Some high energy responses—namely, the maintenance of pinnules of *Mimosa* in the open position, and the extension growth of stems of *Hyoscyamus niger*, *Beta vulgaris*, and *Spinacea oleracea*—have been shown⁴⁰ to have sharp action maxima at 720 nm even when the ratio of P_{FR} to P_R is maintained at a high value by supplementary irradiation with red light. Borthwick *et al.*⁴⁰ have used these examples to extend and complicate the Hartmann hypothesis by suggesting that P_{FRX} (Fig. 2) is dissociated by light with an action maximum at 720 nm but with very little effect in the red regions. This assumption would explain the exceptions to Hartmann's hypothesis, but is not supported yet by any direct evidence about the nature of P_{FRX} .

To sum up, phytochrome is probably located in certain critical membranes, where it is likely to be orientated in a specific dichroic manner. Photoconversion leads to a change in the dichroic orientation of the chromophore and to a change in the tertiary structure of the molecule. P_{FR} is subject to a transformation to a photochemically inactive, but otherwise unknown, form, while P_R may be resynthesized in response to a fall in the levels of total phytochrome. There is evidence that P_R and P_{FR} exist in two forms, a bulk, or "nonsense", form that is photochemically active but physiologically inactive in etiolated tissues, and a minor component that is physiologically active probably in both etiolated plants and those grown in the light. Certain phenomena regulated by phytochrome illustrate a very rapid action of the pigment,

while others demand a series of partial reactions possibly involving the synthesis of enzymes. The far-red sensitive high energy reactions seem in most cases to be explainable in terms of phytochrome. In spite of these advances, many problems still hinder understanding of phytochrome action. Basic questions are involved in the explanation of the specificity of phytochrome responses and their amplification. More challenging problems, perhaps, lie in the nature of phytochrome in plants grown in the light, the role of the decay process, and the explanation of the intensity dependence of the high energy reaction.

Model for Action

Some of these problems can be accounted for satisfactorily by the simple but speculative model presented in Fig. 3. This model is an attempt to rationalize the existing information on phytochrome action and to provide, in particular, a basis for the rapid action and intensity dependence. Phytochrome is thought to exist as orientated molecular arrays in certain critical membranes and its primary action is to transfer an important metabolite (X) from one side of the membrane to the other. This transfer could be driven directly by the conformational changes associated with the photoconversion of P_R to P_{FR} . This assumption would provide a simple explanation for the intensity dependence of the high energy reaction—the higher the intensity of irradiation with wavelengths absorbed by both P_R and P_{FR} , the greater the rate of cycling between P_R and P_{FR} . As long as the mechanisms for the utilization of X on one side of the membrane and its availability on the other side are not rate limiting, the overall rate of reaction will be determined by the rate of cycling and thus by the light intensity. It is not necessary to implicate phytochrome decay to account for the action spectrum of the prolonged-irradiation, high-energy reaction. Because P_R or one of its early photoproducts is assumed to bind to X^1 , maximum rate of transfer might be expected to occur at wavelengths which maintain a high proportion of P_R but still allow sufficient absorption by both P_R and P_{FR} for cycling to occur. For short term irradiations of tissues fully grown in the dark, on the other hand, maximum effect would occur with wavelengths which convert the maximum amount of P_R to P_{FR} . Long term reversibility of events potentiated by a single red irradiation may be due to the utilization of X being limited by the availability of the metabolite or metabolites with which it interacts (A in Fig. 3). On the other hand, the rapid action effects I have described could be due to the immed-

iate interaction of a small part of X^0 with the limited amount of A available, thus producing a small, non-photo-reversible developmental response.

In common with other hypotheses of phytochrome action, this model does not account for the specificity of response, which must reside in the particular state of the genetic control systems in the target cells. Amplification of the response can be ascribed to the synthesis or activation of enzymes caused by the interactions of X once released from its sequestration.

The salient feature of this model is the suggestion that the function of phytochrome is to act as a specific permease for an important metabolite, perhaps a hormone, and that this action is driven directly by the photoconversions. I should like to stress that this model is completely speculative and is not supported by any direct evidence except in so far as it accounts for several of the problems of phytochrome action. The only possible justification for presenting it here is that it may stimulate investigations designed to determine its validity—or otherwise. I hope this will be the case.

I thank Professor P. F. Wareing and Professor M. B. Wilkins for commenting on the manuscript of this article.

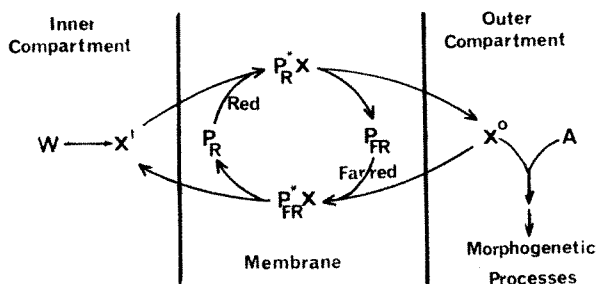


Fig. 3. The speculative model of phytochrome action proposed in the text. P_R^+ and P_{FR}^+ are immediate photoproducts of P_R and P_{FR} ; X^1 represents the pool of X inside the membrane-bound compartment whilst X^0 is the pool of X outside the membrane; A represents one or more metabolites, outside the membrane, with which X interacts, thus initiating the sequences of reactions leading to the morphogenetic processes; W represents the precursors of X . The scheme should not be taken to indicate that phytochrome molecules move within the membrane, rather that the binding sites for X are anisotropic, leading to binding of X on one side of the membrane and its release on the other side. See text for details.

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Citation Indexing for Studying Science

by

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By revealing who has really influenced the course of science the *Science Citation Index* seems to be a valuable sociometric tool for historians and sociologists.

By writing the *Double Helix*¹, Watson laid to rest the absurd notion that scientists have less desire for reputational immortality than other humans. That this belief could have existed at all is only a sign that the efforts of scientists to achieve fame and fortune are necessarily less obvious than those of athletes and politicians. We are thus confronted by a situation wherein those scientists who deserve (and want) recognition cannot always be easily identified, even by their peers. It seems likely, then, that social scientists—whose role is to tell it like it is—will begin to play a larger part in identifying those scientists who have had or will have a major impact on their fields. Indeed, a new breed of sociometrist is developing, called the scientist of science², that is concerned chiefly with the historical, sociological, economic and behavioural study of science and scientists.

Unfortunately, the measurement of science will not become more precise, even though there is a specialized group doing the measuring, unless more effective measuring techniques are developed and used. Most evaluation procedures available to sociometrists are not only slow and costly, they are also tedious. Such practices as counting the number of papers published have been used because truly objective methods were not available. The exponential growth of scientific research and the increasing number of scientists only make matters worse. It is in meeting this need for an effective, efficient, and unobtrusive³ sociometric tool that citation indexing may find its most important application.

A citation index is an ordered list of cited articles, each accompanied by a list of citing articles. The citing article is identified as a source, the cited article as a reference⁴. The *Science Citation Index* (SCI), published by the Institute for Scientific Information, is the only regularly issued citation index in science. It is prepared by computer and provides an index to the contents of every issue published during a calendar year of more than 2,000 selected journals. Journals covered by the index are chosen by advisory boards of experts in each of the topics represented and by large scale citation analyses.

The entry for a cited article (reference) contains the author's name and initials, the cited reference year, and the publication name, volume, and page number. Under the name of each cited author appears the source article citing this work. This line is arranged by citing author's name, publication, type of source item (article, abstract, editorial and so on), citing year, volume, and page. The searcher starts with a reference or an author he has

identified through a footnote, book, encyclopaedia, or conventional word or subject index. He then turns to the *Citation Index* section of the SCI and searches for that particular author's name. When he has located the name, he checks to see which of several possible references fits the particular one he is interested in. He then looks to see who has currently cited this particular work. After noting the bibliographic citations of the authors who are citing the work with which he started, the searcher then turns to the *Source Index* of the SCI to obtain the complete bibliographic data for the works which he has found.

After finding several source articles, the searcher can use the bibliographies of one or several of these as other entries into the citation index; this process is called "cycling". Since authors frequently write more than one closely related paper, additional articles by the author of the starting reference can also be used as entry points to the index.

Basically, then, the SCI does two things⁵. First, it tells what has been published. Each annual cumulation cites between 25 and 50 per cent of the 5 to 10 million papers and books estimated to have been published during the entire history of science. Second, because a citation indicates a relationship between a part or the whole of a cited paper and a part or the whole of the citing paper, the SCI tells how each brick in the edifice of science is linked to all the others⁶. Because it performs these two fundamental functions so well, important applications for the SCI have been found in three major areas: library and information science, history of science, and the sociology of science.

The SCI was originally designed to be a retrieval tool for use in library and information science work⁷. It has served this purpose very well. The unique retrieval effectiveness of the SCI has already been reported by several investigators⁸. The worldwide adoption of SCI in its short history confirms its ability to augment traditional indexing methods.

Uses in Historical Research

Besides retrieval, other uses for the SCI in library and information science are emerging. Because well over 20 million bibliographic citations have been extracted from more than 1,500,000 source documents, the SCI data base can be utilized to provide definitive studies of journal-to-journal relationships. A recent study by Martyn⁹ illustrated how the SCI data base could be used

to rank British scientific journals and pick out the effective "hard core" of literature. Soon, the Institute for Scientific Information will publish a statistical compilation which will show how often each of 2,000 journals cite one another. This *Source Journal Citation Index* will be complemented by the *Reference Journal Citation Index*, which will show how often each of these 2,000 journals cites any of more than 25,000 other journals.

The suggestion for using citation indexing for historical research came as early as 1955¹⁰. Dr Gordon Allen gave great impetus to this idea when he constructed a bibliographic citation network diagram in 1960 (personal communication from G. Allen). In 1964 the practical methodology was developed to permit the use of citation indexing in sociological and historical research to identify key events, their chronology, their interrelationships, and their relative importance¹¹.

Fig. 1 shows the application of SCI data to create a graphic aid to the study of the history of science. By examining the interconnecting links of scientific events shown in the citation network, it is possible to observe historical and sociological processes at work. It is also easy to identify the nodal publications in the citation network, that is, those that are cited most by others, those that have had the most impact. From Fig. 1 it would be quite reasonable to conclude that whoever published paper number 2 had considerable impact on research involving nucleic acid staining. It is at this point that the SCI begins to serve as an objective sociometric tool; it begins to show who has truly influenced the course of science.

In addition to identifying individuals whose work has had impact on a branch of science, carefully constructed citation networks can help disprove certain prevailing scientific myths. For example, it is commonly believed that Gregor Mendel's breakthrough paper on genetics

was ignored by the scientific community from the time it was presented in 1865 until it was "rediscovered" in 1900. The citation network in Fig. 2 shows, however, that not only was Mendel's work not ignored, but that it was actually cited by at least four different people before 1900. Mendel's work was even cited in an article on "hybridism" in the ninth edition of the *Encyclopaedia Britannica*. One could hardly call that being ignored.

Citation networks can also bring into focus anomalies in the history of scientific development. In Fig. 2, for example, why did Darwin's 1876 paper cite Hoffman but not Mendel? Certainly this is unusual, since Hoffman's paper cites Mendel five times. Inconsistencies like these are clearly identified in citation networks and give impetus and assistance to all types of important historical research.

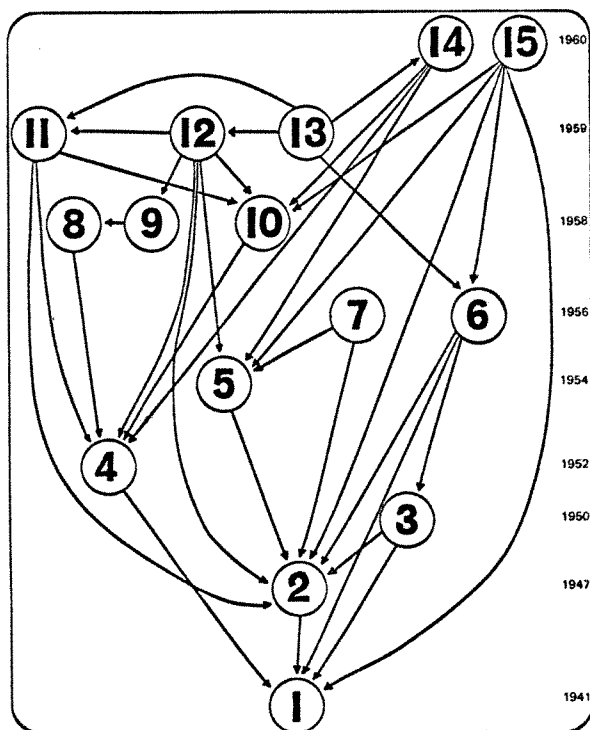


Fig. 1. Citation network of fifteen articles on nucleic acids.

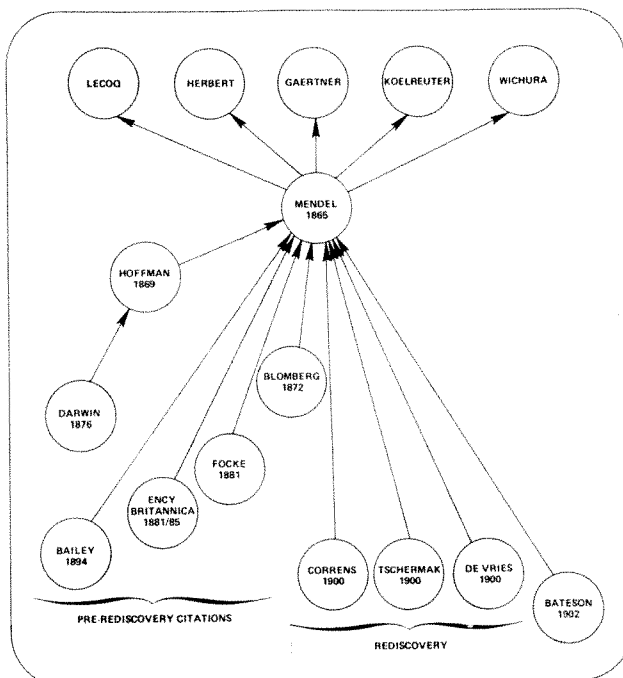


Fig. 2. Citation network showing citations to Mendel before alleged "rediscovery". This was discussed by Zirkle in ref. 11a.

The citation networks shown were produced manually, but further work¹² indicates that such diagrams can be assembled automatically using large computer memories and programs for iterative display of appropriate data. This means that in the near future a historian or sociometrist will be able to sit before a computer console and specify some starting point—a person, a word, a citation, or a place¹³. He will then ask the computer to display a list of pertinent papers. The computer will respond by drawing or displaying a historical road map which will show not merely a list of papers and books, but also a graphical approximation of the history of that subject.

It was a logical step to progress from using the SCI as a sociometric tool in historical contexts to using it to measure current scientific performance¹⁴. Bayer¹⁵, Martino¹⁶, and others have already reported that valid correlations can be obtained between individual performance and citation counts. Perhaps the most dramatic indication of the sociometric power of the SCI was the forecast made in 1968 of those who would win Nobel prizes in 1969¹⁷.

	RANK	AUTHOR	TOTAL TIMES CITED	RANK	AUTHOR	TOTAL TIMES CITED
	1	LOWRY OH	2921	26	ELIEL EL	721
	2	CHANCE B	1374	27	STREITWIESER A	717
	3	LANDAU LD	1174	28	MULLIKEN RS	712
	4	BROWN HC	1150	29	JACOB F	711
	5	PAULING L	1063	30	BORN M	710
Nobel Prize in Physics 1969	6	GELLMANN M	942	31	BRACHET J	706
	7	COTTON FA	940	32	WINSTEIN S	702
	8	POPLE JA	933	33	ALBERT A	687
	9	BELLAMY LJ	906	34	LUFT JH	674
	10	SNEDECOR GW	904	35	DEDUVE C	673
	11	BOYER PD	893	36	VONEULER US	668
	12	BAKER BR	876	37	FIESER LF	666
	13	KOLTHOFF IM	853	38	HUISGEN R	661
	14	HERZBERG G	842	39	NOVIKOFF AB	655
	15	FISCHER F	826	40	GOODWIN TW	643
	16	SEITZ F	822	41	BARTON DHR	632
	17	DJERASSI C	801	42	FISHER RA	631
	18	BERGMAYER HU	754	43	BATES DR	627
	19	WEBER G	750	44	FLORY PJ	626
	20	REYNOLDS ES	748	45	STAHL E	626
	21	MOTT NF	741	46	DEWAR MJS	619
	22	ECCLES JC	737	47	GILMAN H	618
	23	FEIGL F	729	48	FOLCH J	618
	24	FREUD S	727	49	DISCHE Z	614
	25	PEARSE AGE	726	50	GLICK D	609

Fig. 3. Fifty most cited authors for 1967, ranked according to total number of times cited.

Predicting Nobel Prize Winners

By using the SCI data base, it was possible to list the fifty most cited authors for 1967 as shown in Fig. 3. Two of the 1969 Nobel prize winners, Derek H. R. Barton and Murray Gell-Mann, appeared on the list. There are about one million scientists in the world and so to produce a list of fifty that contains two Nobel prize winners is no small achievement. It is especially impressive when one considers that the approach is based on a purely objective method which does not require a personality appraisal or a reading of the works by these men.

Although forecasting Nobel prize winners is an interesting exercise, the ability of the SCI to identify those individuals who will make a major impact on science has more practical social and economic consequences. Research administrators in academic, industrial, and government organizations have frequently indicated the need for a tool for identifying such people. Increasingly scarce intellectual and financial resources for supporting research could be managed more efficiently with such an identification tool. Creative people could be identified much earlier in their careers so that they could benefit from special training. Prizes, grants, fellowships and other forms of recognition could be awarded without the wasteful in-fighting and manoeuvring among scientists described by Watson¹.

Another problem facing research administrators is how to determine the directions research should take in the future. The recent summary of the difficulties involved in selecting lunar experiments for the Apollo program¹⁸ is a good current example of this type of dilemma.

In this kind of situation, imaginative use of the SCI data base might contribute to a solution. In the near future ISI will publish what should prove to be a valuable forecasting tool. This will be a regularly published list of the 20,000 papers which are cited most in a given year.

Proper analysis of this information could be a giant step forward in identifying "where the action is" (or should be) in the area of scientific research.

When the *Science Citation Index* was first proposed, its major objective was to break the so-called subject index barrier¹⁹. Out of this bibliographic experiment has evolved a historiographic and sociometric tool of major importance. Like most other scientific discoveries, this tool can be used wisely or abused. It is now up to the scientific community to prevent abuse of the SCI by devoting the necessary attention to its proper and judicious exploitation.

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Role of Methionine in the Initiation of Haemoglobin Synthesis

by

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The α and β chains of rabbit globin are initiated by the incorporation of a methionine residue in the N-terminal position. This methionine is removed during the early stages of chain growth.

FOR a long time it has not been clear whether a special species of tRNA is required for the initiation of protein synthesis in eukaryotic cells¹. In the particular case of haemoglobin synthesis there have been a number of conflicting reports. On the one hand, it has been claimed that short nascent α chains have a modified and as yet unidentified N-terminal residue². On the other hand, the results of incorporation experiments using tRNA carrying modified valine residues have been taken as an indication that no special initiating tRNA exists other than the valyl-tRNA translating the N-terminal codon³⁻⁵. Consistent with this are the reports that newly synthesized haemoglobin and also the nascent protein chains on reticulocyte ribosomes have valine as the only detectable N-terminal residue⁶⁻⁷.

We report here that methionine is the N-terminal residue of short nascent protein chains bound to rabbit reticulocyte ribosomes. We propose that methionine is incorporated into the N-terminal position of both α and β chains of rabbit haemoglobin during the initiation process of protein synthesis and is removed during the early stages of peptide chain growth.

Isolation of Ribosomes in a Post-initiation State

Our approach was to examine the short nascent peptides found on ribosomes shortly after these ribosomes had initiated new chains. The ribosomes were synchronized in a pre-initiation state by using sodium fluoride to inhibit the initiation of new chains while pre-existing nascent protein was completed⁸⁻¹⁰. The NaF was then removed, thus allowing the initiation of new nascent chains, but the growth of these chains was restricted by the use of inhibitors (for example, sparsomycin). Rabbit reticulocytes from phenylhydrazine treated animals were washed and incubated with 10 mM NaF at 37° C for 30 min in the complete medium containing amino-acids and ferrous ions¹¹. Following this pre-incubation a stroma-free lysate was prepared, gel-filtered on 'Sephadex G-25', and incubated at 37° C in the following conditions: 70 mM KCl, 2.7 mM magnesium acetate, 5 mM 2-mercaptoethanol, 1.8 mg/ml. creatine phosphate, 50 μ g/ml. creatine phosphokinase, 1.0 mM ATP, 0.1 mM GTP and 25 mM Tris-HCl (pH 8.1 at 0° C and pH 7.8 at 37° C), together with the radioactive amino-acid required and complementary unlabelled amino-acids. The incorporation of radioactive amino-acids into protein in such lysates was 80-95 per cent inhibited if 10 mM NaF was added, and was therefore largely dependent on the initiation of new nascent chains¹⁰.

To restrict the growth of these newly initiated chains, sparsomycin¹² was added at a final concentration of 40-140 μ M. After an incubation period of 5-20 min, the ribosomes were isolated by centrifugation at 100,000g through 1 M sucrose containing the buffered salts medium (lacking 2-mercaptoethanol) used in the incubation. The labelled material bound to these ribosomes had the following properties regardless of the labelled amino-acid used: (1) it was precipitated by cetyl trimethyl ammonium

bromide (CTAB), which only precipitates peptides and proteins if they are covalently linked to RNA (unpublished observations of R. J. J.); (2) it has a high reactivity towards puromycin as assayed by precipitation with CTAB; (3) labelled material is almost exclusively (greater than 85 per cent) extracted into the aqueous phase on preparation of the RNA from the ribosomes by phenol extraction at pH 5.2; (4) the puromycyl peptides are extracted to a high degree (60-70 per cent) into ethyl acetate at pH 10 (ref. 13).

Because the normal random length nascent protein formed in the absence of inhibitors was similar with respect to the first two properties, but markedly different in the last two, the product formed in the presence of sparsomycin must be almost exclusively short nascent peptides. The composition and yield of this material were estimated using various individual labelled amino-acids, or a ¹⁴C-amino-acid mixture, followed by hydrolysis of the peptides and separation of the constituent amino-acids. At high concentrations of sparsomycin (140 μ M), methionine and valine were the only amino-acids present in these short peptides. At lower levels (50 μ M), approximately equimolar amounts of methionine, valine and leucine were estimated; and in addition proline, alanine, serine and histidine were found. The yield of methionine residues per ribosome was the same at the two concentrations of sparsomycin. The fraction of ribosomes synthesizing these short peptides was calculated to be 10-15 per cent, which is close to the proportion expected to be active immediately after the removal of NaF⁸⁻¹⁰. These results indicate that the product resembles the N-terminal segments of the α and β chains of rabbit globin except for the presence of a methionine residue (see Fig. 1).

α chain:	Val-	Leu-	Ser-	Pro-	Ala-	Asp-	Lys-	Thr...
	1	2	3	4	5	6	7	8
β chain:	Val-	His-	Leu-	Ser-	Ser-	Glu-	Glu-	Lys...
	1	2	3	4	5	6	7	8

Fig. 1. N-terminal sequences of the α and β chains of rabbit haemoglobin. The sequence of the α chain is that determined by von Ehrenstein¹⁴ and of the β chain by Braunitzer, Best, Flamm and Schrank¹⁴.

Sequence Analysis of the Short Peptides

The homogeneity of the N-terminal sequences of the peptides formed at 40-50 μ M sparsomycin was examined by Edman degradation of the unfractionated material (Fig. 2). The recoveries of radioactivity for each labelled amino-acid were comparable and not a source of selective error. When the peptides were labelled with ³⁵S-methionine, approximately 75 per cent of the radioactivity was released in the first round and the remaining 25 per cent in the second round (Fig. 2). It seems that the efficiency of each cycle of the Edman degradation is approximately 75 per cent in these conditions, and that all the methionine is N-terminal. This view is justified, for Edman degradation of individual peptides separated by electrophoresis gave results identical to the unfractionated material, and no peptide could be found which yielded methionine chiefly in the second round of degradation. Valine was

obtained principally in the second round of degradation, and the remainder was released in the third round (Fig. 2). By the same reasoning, valine was assigned exclusively to the second position. If the same efficiency is maintained in the third cycle of degradation, about 60 per cent of the total leucine and 30 per cent of the histidine occurred in the third position. The proportion of histidine in this position may be seriously underestimated, however, for the extraction of the phenylthiohydantoin derivative of histidine into butyl acetate is not efficient¹⁶.

Although these results show that methionine was found in the N-terminal position and valine in the second position, they do not rule out the possibility that two classes of peptides exist, one with the N-terminal sequence Met-X . . . and the other with the sequence Y-Val This possibility was rigorously excluded by Edman degradation of peptides from which the methionine residue had been cleaved specifically by treatment with cyanogen bromide¹⁷. The efficiency of this cleavage was rather low, but there was a close correlation between the efficiency of cleavage and the proportion of ¹⁴C-valine released one round earlier than normal in the Edman degradation (Fig. 3). Because equimolar quantities of methionine and valine occur in these peptides (see above), this proves unambiguously that methionine and valine residues exist in peptides of sequence Met-Val This sequence does not occur in either chain of completed rabbit haemoglobin¹⁴.

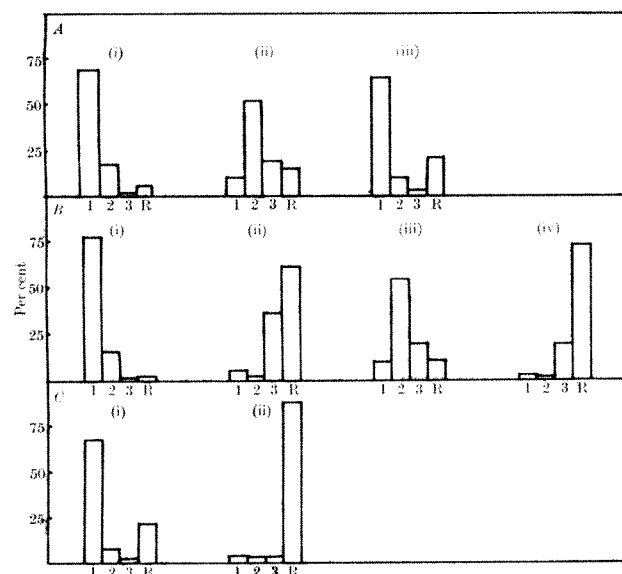


Fig. 2. Edman degradation of short peptide material. For A and B, the labelled peptides were obtained by the following procedure: incubation of lysates from NaF treated cells with the radioactive amino-acid specified, isolation of the ribosomes, extraction of the ribosomal RNA, and treatment with 1 per cent trimethylamine to strip the peptides from rRNA (see text). The three stage Edman degradation was done by the method of Gray¹⁵. After each cycle the released phenylthiohydantoin was extracted into butyl acetate. The butyl acetate extracts and the residue remaining after the third step were dried down, dissolved in acetone and formic acid respectively, and transferred to an aqueous scintillator¹¹. The radioactivity released at each step and that remaining in the residue (R) are expressed as a percentage of the radioactivity recovered. Recoveries are expressed as a percentage of the input radioactivity; it was not possible to estimate the recoveries of the ³H-amino-acids. A, Labelling conditions: incubation for 8 min with 50 μ M sparsomycin (except in iii), and either L-³⁵S-methionine (9 Ci/mole; 3.0 μ Ci/ml. final concentration) or L-¹⁴C-valine (270 mCi/mole; 1.5 μ Ci/ml.). (i) ³⁵S-methionine peptides (recovery 91 per cent); (ii) ¹⁴C-valine peptides (recovery 90 per cent); (iii) ³⁵S-methionine peptides labelled in absence of sparsomycin (recovery 96 per cent). B, Labelling conditions: incubation for 10 min with 40 μ M sparsomycin and either ³⁵S-methionine (4.6 μ Ci/ml.) and L-4,5-³H-leucine (24.3 Ci/mole; 8.0 μ Ci/ml. final concentration), or ¹⁴C-valine (1.7 μ Ci/ml.) and L-2,5-³H-histidine (7.4 Ci/mole; 8.0 μ Ci/ml.). (i) ³⁵S-methionine distribution (recovery 81 per cent); (ii) ³H-leucine distribution; (iii) ¹⁴C-valine distribution (recovery 90 per cent); (iv) ³H-histidine distribution. C, Short peptide material isolated from ribosomes as given above, except that the ribosomes were from whole cells which were incubated with labelled amino-acids in conditions of tryptophan starvation (see text). (i) ³⁵S-methionine labelled peptides (recovery 96 per cent); (ii) ¹⁴C-amino-acid labelled peptides (recovery 70 per cent).

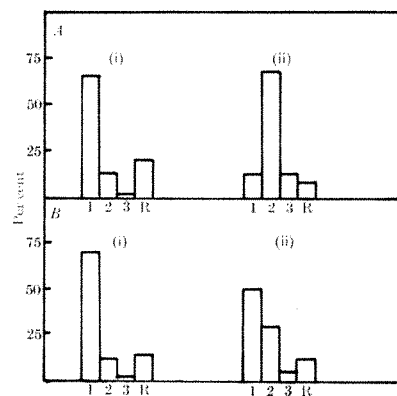


Fig. 3. Edman degradation of labelled peptides after cyanogen bromide treatment. The peptides were obtained by the method described in the text and in Fig. 2. Labelling conditions were 10 min incubation with 40 μ M sparsomycin and either (i) ³⁵S-methionine (4.6 μ Ci/ml.) or (ii) ¹⁴C-valine (1.7 μ Ci/ml.). The peptides were incubated for 24 h at room temperature in (A) 0.4 ml. 70 per cent formic acid or (B) 0.4 ml. 70 per cent formic acid containing 5 mg cyanogen bromide¹⁷. After being vacuum dried three times, the peptides were dissolved in 0.1 ml. 0.01 M HCl and extracted three times with butyl acetate to remove ³⁵S-labelled by-products which might interfere. The final aqueous phase was sampled to assay radioactivity, dried and subjected to Edman degradation as described in Fig. 2. The loss of radioactivity on treatment with cyanogen bromide was 51 and 63 per cent (duplicate samples) in the case of ³⁵S-methionine-labelled peptides, and 7 per cent for ¹⁴C-valine labelled peptides. From these figures an efficiency of cyanogen bromide cleavage of 57 per cent was calculated. The recoveries of radioactivity in the Edman degradation were all greater than 80 per cent. A, Sham treated controls: (i) ³⁵S-methionine distribution; (ii) ¹⁴C-valine distribution. B, Cyanogen bromide treated peptides: (i) ³⁵S-methionine distribution (labelled material remaining after cyanogen bromide treatment.) (ii) ¹⁴C-valine distribution.

Electrophoresis of Short Peptide Products

The peptides stripped from the RNA by exposure to 1 per cent trimethylamine were separated by electrophoresis at pH 3.5, and examined for ³⁵S-methionine distribution by autoradiography (Fig. 4). Some heterogeneity was caused by methionine existing in different states of oxidation, as demonstrated by the simplification obtained by oxidation with air or performic acid before electrophoresis. For example, air oxidation¹⁸ of the material A eliminated the more rapidly migrating components of spots 2 and 4 (see Fig. 4D).

Heterogeneity in the pattern was also inversely related to the concentration of sparsomycin present in the incubation (see Fig. 4A-C). At very high concentrations only two products are formed. One of these is free methionine and its oxidation products (spot 4, Fig. 4). The other (spot 2) had identical mobilities with chemically synthesized methionyl-valine on electrophoresis at pH 3.5 and on chromatography in the system of Waley and Watson¹⁹. As further proof of the structure, it was isolated, labelled with ³⁵S-methionine and ³H-valine and subjected to one cycle of Edman degradation. Of the ³H-valine radioactivity in the aqueous phase after this one cycle, 80-85 per cent was recovered as free valine on electrophoresis at pH 3.5 and then perpendicularly at pH 2.1. The corresponding peptide (spot 2) formed at lower concentration of sparsomycin (Fig. 4B) has also been identified as Met-Val by the same procedure.

A comparison of the peptide products formed at 140 μ M and 50 μ M sparsomycin revealed, first, that the dipeptide Met-Val was the only peptide product formed at the higher concentration, and is one of the peptides found at the lower level of sparsomycin; second, that the yield of methionine residues per ribosome was the same in the two conditions; third, several species of peptides containing methionine were formed at the lower concentration of sparsomycin (Fig. 4B), but Edman degradation of the unfractionated peptides showed that these have the N-terminal sequence Met-Val

As might be expected, the concentration of sparsomycin therefore determined the size of the peptide products but not the N-terminal sequences found. The only peptide

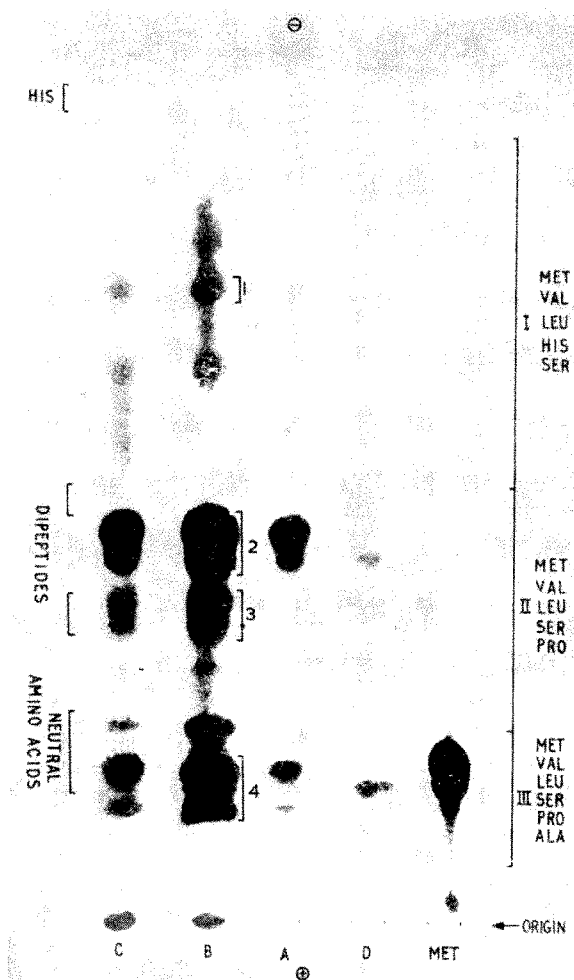


Fig. 4. Electrophoresis of short peptides labelled with ^{35}S -methionine. The peptides were obtained by the following procedure: incubation of a lysate from NaF treated cells for 10 min with ^{35}S -methionine (1.7 $\mu\text{Ci}/\text{ml}$, final concentration) and the sparsomycin concentrations given below, isolation of the ribosomes, extraction of the ribosomal RNA and treatment with 1 per cent trimethylamine to strip the peptides from tRNA. These peptides and a ^{35}S -methionine marker were electrophoresed at pH 3.5 for 1 h at 3 kV on Whatman '3MM' paper. The autoradiogram obtained after 6 days exposure is shown. The positions of neutral amino-acids, histidine and two neutral dipeptides (alanyl-leucine and leucyl-tyrosine) were detected by ninhydrin staining. The sparsomycin concentrations used were (A) 140 μM , (B) 60 μM and (C) 20 μM and the amounts of material applied were 5,350 c.p.m., 30,860 c.p.m. and 10,360 c.p.m. respectively. (D) is 1,700 c.p.m. of the same material as (A) but subjected to air oxidation¹⁸ before electrophoresis. The amino-acids present in the peptides in groups I, II and III were determined separately using pooled peptides labelled with a mixture of ^{14}C -amino-acids at 50 μM sparsomycin. The eluted peptides were hydrolysed in 6 M HCl for 24 h at 110° C; the hydrolysates were mixed with unlabelled amino-acids and subjected to electrophoresis at pH 2 for 25 min at 5 kV followed by descending chromatography in *tert*-amyl alcohol (pH 6.5). All ninhydrin positive spots were eluted and counted by liquid scintillation counting¹¹. The radioactive amino-acids detected in each group are shown in the figure: equimolar amounts are not necessarily present. Compositions were also determined by electrophoresis of peptides labelled with ^{35}S -methionine and ^3H -valine, ^3H -leucine or ^3H -histidine at 50 μM sparsomycin, followed by elution of the various spots and liquid scintillation counting.

product formed at high levels, the dipeptide Met-Val, has been lengthened into oligopeptides of sequence Met-Val . . . under the less stringent restriction of chain growth. Edman degradation of the unfractionated material has demonstrated that these oligopeptides include peptides which have the sequences Met-Val-Leu . . . and Met-Val-His The former must account for about half of the material, for the unfractionated material contained leucine in equimolar amounts with respect to methionine and valine, and 60 per cent of the leucine was found in the third position. The proportion of peptides with the sequence Met-Val-His . . . could not be determined.

The composition of the separated peptides or groups of peptides was examined as described in Fig. 4. All the peptides of high mobility (group I) contained histidine at the same ratio of methionine to histidine, and because histidine occurred only in this group of peptides, at least some must have the sequence Met-Val-His . . . established above. Some of these peptides contained leucine (for example, spot 1) and serine. The ^{35}S -methionine label in all of them, and the ^3H -leucine label in spot 1, migrated towards the cathode at pH 6.5. All the peptides of lower mobility than the dipeptide Met-Val contained leucine, and some also had proline and alanine residues. Edman degradation showed that the leucine in this group of peptides was almost exclusively in the third position. These peptides therefore largely account for the half of the material which has been shown to have the sequence Met-Val-Leu and the mobility of spot 3 suggests that it may be the tripeptide Met-Val-Leu.

An approach comprising Edman degradation of the unfractionated peptides and certain groups of separated peptides, together with analysis of the composition of separated peptides, has therefore established that the products formed at 50 μM sparsomycin consist of free methionine, the dipeptide Met-Val and two main classes of oligopeptides: (a) those which lack histidine and have the sequence Met-Val-Leu . . . and therefore probably represent α chain peptides, and (b) those which have the sequence Met-Val-His . . . but lack proline and alanine, and therefore seem to be β chain peptides (see Fig. 1).

N-Terminal Methionine of Nascent Protein from Intact Cells

We next examined whether methionine was in the N-terminal position of short nascent globin chains synthesized in intact cells. To obtain ribosomes carrying mainly short nascent chains, the cells were starved of tryptophan by pre-incubation at 37° C for 40 min in the complete medium lacking tryptophan¹¹. They were then incubated for 4 min with either ^{14}C -amino-acid mixture or ^{35}S -methionine, before arresting protein synthesis by the addition of cold saline containing actidione (1 mg/ml.)¹¹. The ribosomes were prepared as described. The nascent protein was equally reactive towards puromycin, whether labelled with ^{35}S -methionine or with ^{14}C -amino-acids. When the short peptides were isolated by extraction of the RNA from the ribosomes, 31 per cent of the ^{35}S -methionine was recovered as opposed to 19 per cent of ^{14}C -amino-acid label. Edman degradation of this short peptide material showed that most of the methionine was in the N-terminal position (Fig. 2), and electrophoresis at pH 3.5 showed that it was not in the form of free methionine but was in peptides which gave a pattern similar to that of the product formed *in vitro*. It was calculated that not less than 25 per cent of the total ^{35}S -methionine in this nascent protein labelled *in vivo* was in the N-terminal position.

Fate of the N-Terminal Methionine Residue

Because valine is found in the N-terminal residue of most nascent chains⁶⁻⁷, the methionine in the N-terminal position of short chains must be removed during the early stages of peptide growth. To study this we incubated ribosomes with cell sap in conditions for extension and completion of the chains, using ribosomes which carried exclusively short labelled nascent chains (Fig. 5). The radioactivity of peptidyl tRNA (CTAB-precipitable radioactivity) and of long peptides (acid-precipitable radioactivity after NaOH treatment) was assayed. The excess of CTAB-precipitable radioactivity over acid-precipitable radioactivity at the start of the incubations represents short peptides attached to RNA. The decrease in CTAB precipitable radioactivity during the incubation represents the release of the protein from RNA presumably as completed chains. The increment in acid-precipitable

radioactivity must represent the entry of label from short peptides into long peptides, that is, extension of short nascent chains. Valine and other amino-acids were transferred with reasonable efficiency into stable long peptides, but the transfer of the methionine was poor (Fig. 5), although it did appear temporarily in the long peptides after short incubation periods. Because these changes were inhibited by sparsomycin but not by NaF they are dependent on protein synthesis but not on initiation of new peptide chains. The methionine residue, which occupies only the N-terminal position of these short nascent chains, is therefore conserved in the earliest stages of chain growth at least to the point when the polypeptide becomes acid precipitable, and then is removed soon afterwards.

The same experiment was performed using the ribosomes which carried nascent chains labelled with ^{35}S -methionine during incubation of intact cells. Again there was an early but transient increment in the acid-precipitable radioactivity showing the retention of the N-terminal methionine for only a limited period of peptide chain growth.

When the yield of the various tryptic peptides obtained from nascent globin was measured¹¹, $\alpha\text{T}1$ and $\beta\text{T}1$ were found in especially low yield if the ribosomes were situated chiefly near the initiation site (that is, in tryptophan starved cells), but in the expected amounts if the ribosomes were chiefly at the distal end of the mRNA (that is, shortly after the addition of NaF). The presence of N-terminal methionine on short nascent chains accounts for this previously unexplained deficit dependent on the average chain length. Because the yield of these two peptides was 10–15 per cent lower than expected when the ribosomes were randomly distributed¹¹, N-terminal methionine must be removed when the nascent chains attain a length of fifteen to twenty residues. This estimate is consistent with the retention of N-terminal methionine when relatively short synthetic polynucleotides were used as templates²⁰. Because a length of nascent protein corresponding to about thirty residues is protected against external proteolytic enzymes^{3,21}, it is possible that the methionine aminopeptidase is a ribosomal protein. On the other hand, we find that reticulocyte ribosomes do not remove N-terminal methionine from puromycin peptides (unpublished observations).

Although the sequential use of NaF followed by sparsomycin provides the most convenient system in that a good yield of a relatively homogeneous short peptide product was obtained, there is a risk that such inhibitors may induce artefacts. But the use of 5'-guanylyl-methylene-diphosphonate (in the absence of GTP), or of diphtheria toxin and NAD, instead of sparsomycin gave a material which was similar in composition, electrophoretic mobility and in having the N-terminal sequence Met-Val... but in reduced yield. If no inhibitor of chain elongation was used, the ribosomes nevertheless carried some short peptides with N-terminal methionine (Fig. 2). Finally, the observation that methionine is in the N-terminal position of short chains labelled in the intact cell validates the results obtained with the *in vitro* system.

Most previous studies of initiation in reticulocytes do not conflict with our conclusion. Valine has been reported to be the N-terminal residue of nascent globin chains⁵⁻⁷, as we have ourselves found by the same methods. But the slightly lower yield expected if 10 per cent of the chains have an N-terminal methionine residue has actually been noted by others^{5,7}. The apparently contradictory claim that amino-acids other than valine were not found in the N-terminal position of nascent chains⁶ can be explained by the absence of ^{14}C -methionine from the ^{14}C -amino-acid mixture used.

The only clear contradiction is the ability of 2-hydroxyisovaleryl-tRNA to insert 2-hydroxyisovaleryl groups into globin presumably in the N-terminal position^{3,4}. Our results imply that initiation by this modified valine

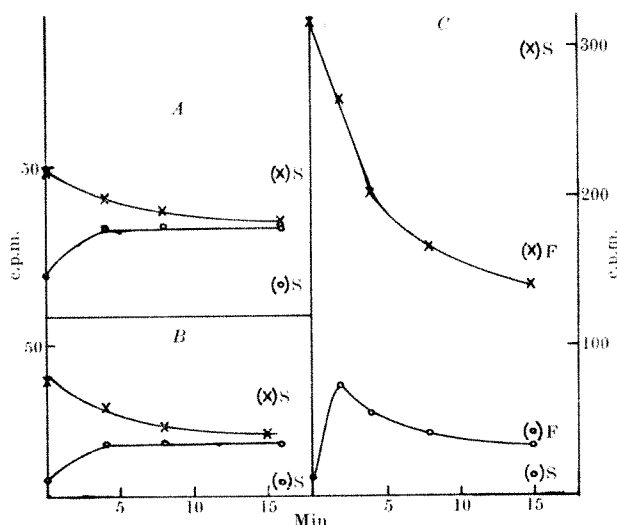


Fig. 5. Re-incubation of ribosomes carrying short nascent peptides. A lysate from NaF treated cells was incubated as given in the text for 8 min with 40 μM sparsomycin and either (A) a mixture of fourteen uniformly ^{14}C -labelled L-amino-acids (2 mCi/mg; 1.4 $\mu\text{Ci}/\text{ml}$, final concentration) plus excess (100 μM) unlabelled valine, (B) ^{14}C -valine (1.5 $\mu\text{Ci}/\text{ml}$) or (C) ^{35}S -methionine (3.0 $\mu\text{Ci}/\text{ml}$). The ribosomes isolated from these incubations (see text) were re-incubated with fresh gel-filtered cell sap (100,000g supernatant) in the conditions given in text except that all twenty amino-acids were present in the unlabelled form. CTAB-precipitable radioactivity ($\times-\times$) was estimated in 0.05 ml. samples by the addition of 1.5 ml. 2 per cent CTAB and 1.5 ml. 0.5 M sodium acetate-acetic acid buffer pH 5.2 containing 1 mg/ml. carrier yeast RNA. The precipitate was filtered on glass fibre filters and washed with water. Acid-precipitable alkali-stable radioactivity ($\circ-\circ$) was measured in 0.05 ml. samples by the addition of excess acid acetone (1 per cent conc. HCl) and isolation of the precipitate by centrifugation. The protein was dissolved and incubated at 37°C for 30 min in 0.3 M NaOH containing the appropriate unlabelled amino-acid at approximately 1 mg/ml., then reprecipitated by the addition of 8 per cent trichloroacetic acid also containing the appropriate unlabelled amino-acid. The precipitate was filtered on glass fibre filters and washed with trichloroacetic acid. The filters were glued to cardboard disks, dried, and radioactivity was counted in a Nuclear Chicago gas flow counter with an efficiency of approximately 22 per cent. Isolated points marked S refer to incubations with 50 μM sparsomycin, and those marked F to incubations with 10 mM NaF. In 20 min incubation with ^{14}C -leucine and nineteen corresponding unlabelled amino-acids in the same conditions, the following increments in acid-precipitable radioactivity were noted: (A) 84 c.p.m., (B) 72 c.p.m., (C) 127 c.p.m. and control untreated ribosomes 765 c.p.m. per A_{260} unit of ribosomes incubated.

residue must be abnormal, and it is therefore of interest that only a small proportion of the chains were initiated in this way³.

Our results provide the necessary evidence that the first of the three models of initiation proposed by Smith and Marcker²² is correct and valid for protein synthesis on natural mRNA: an N-terminal methionine residue is incorporated during the initiation of α and β chains of rabbit globin and is removed during the early stages of chain elongation. This conclusion seems broadly in agreement with forthcoming reports from two independent sources^{23,24}. It remains to be proved whether this mechanism of initiation is generally applicable to all eukaryotic cells. It is of interest that methionine is rarely found in the N-terminal position of completed proteins in eukaryotic cells²⁵, and the initiation of those proteins which have N-terminal methionine—for example, β chains of certain haemoglobins²⁶—clearly deserves special investigation.

The theory of Smith and Marcker²² demands that the methionine residues which initiate rabbit globin chains are unmodified and are furnished by a particular species of tRNA. We shall examine the validity of this proposition elsewhere.

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Transient Incorporation of Methionine at the N-Terminus of Protamine Newly Synthesized in Trout Testis Cells

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Evidence is obtained in another eukaryotic system for the universal role of methionine as the initiating amino-acid in protein synthesis.

N-FORMYLMETHIONYL-tRNA_f was discovered by Marcker and Sanger in 1964 (ref. 1) and since then has been established as the initiator of protein synthesis in bacteria²⁻⁴ and implicated in mitochondrial and chloroplast protein synthesis^{4,5}, the involvement of 70S ribosomes being common to all these systems. The initiation mechanism on the 80S ribosomes in eukaryotic cell cytoplasm is known to involve initiation factors^{6,7} but a role for a specific initiator tRNA has not been demonstrated. Two species of Met-tRNA have been found in the cytoplasm of guinea-pig liver⁸ and yeast⁹; one of these, Met-tRNA₁, can be formylated by purified *Escherichia coli* transformylase in the presence of N¹⁰-formyltetrahydrofolate but no endogenous transformylase activity has been detected in the cytoplasm from which these tRNAs were extracted. Met-tRNA₁ from both systems binds to ribosomes in the presence of the initiator codons AUG and GUG; the yeast Met-tRNA₁ also binds in the presence of UUG and was shown to be active in a cell-free system derived from *E. coli* primed with RNA from bacteriophage f2 (ref. 9). The rapid rate of formation of methionyl-puromycin with the liver Met-tRNA₁ suggested that it was bound at the peptidyl site on the ribosome¹⁰. Met-tRNA₁ therefore resembles *E. coli* Met-tRNA_f, but attempts to demonstrate a formylated Met-tRNA in eukaryotic cell cytoplasm have not been successful.

We discuss here the role of methionine in the biosynthesis of protamine in testis cells of the rainbow trout (*Salmo gairdneri*). Protamines are the basic proteins bound to DNA in the nuclei of sperm cells of most higher organisms. By contrast with mammalian testis, the fish testis develops quite synchronously from a thread-like structure composed chiefly of spermatogonial cells to the large adult organ containing chiefly spermatids and mature sperm. Rainbow trout protamine has a molecular weight close to 5,000 and three components have been purified and sequenced by Ando and Watanabe¹¹. There are 31-32 residues with arginine occupying 21-23 of the positions; proline is N-terminal in each component

and acidic, aromatic and sulphur-containing amino-acids are absent.

Protamine is synthesized in the cytoplasm of testis cells at the spermatid stage of differentiation; this synthesis occurs on 120S disomes composed of 77S ribosomes¹². Newly synthesized protamine is rapidly transported into the nucleus and thence into the chromatin where it replaces the histones in a definite order¹³. The synthesis is very sensitive to inhibition by cycloheximide and is also inhibited by puromycin¹⁴.

Methionine Incorporation

Fig. 1 shows incorporation of [¹⁴C-methyl]-methionine into testis histones and protamine; the latter forms the second major peak in the elution profile. An aliquot of this labelled protamine was electrophoresed in a starch-urea-aluminium lactate gel¹⁵; all of the radioactivity had the same mobility as an unlabelled protamine marker or moved just behind it. Newly synthesized protamine is phosphorylated^{15,17} and is known to run more slowly towards the cathode at the pH of the gel (3.4) than older dephosphorylated protamine¹⁸ which is the predominant form in the marker. It is therefore clear that the ¹⁴C-methyl label is incorporated into protamine.

A second aliquot of the labelled protamine was hydrolysed with 6 M HCl at 110° C for 22 h and the resulting mixture of free amino-acids electrophoresed at pH 1.9; the radioactivity had the same mobility as an unlabelled methionine marker. After performic acid oxidation and electrophoresis at pH 1.9, the label moved with an unlabelled methionine sulphone marker. Thus the ¹⁴C-methyl label must have been incorporated into protamine as intact methionine.

Methionine is N-Terminal

To determine whether the incorporated methionine was N-terminal, Edman degradation and dansylation were performed on purified ³⁵S-methionine labelled protamine. Table 1 shows that 75-90 per cent of the label can be

Table 1. EDMAN DEGRADATION OF ^{35}S -METHIONINE LABELLED PROTAMINE

Exp.	Aqueous phase (c.p.m.)	Ethyl acetate phase	PTH-Methionine (per cent)
1	1,660	14,080	89
2 (a)	5,568	17,561	76
(b)	5,214	13,523	72

Aliquots of ^{35}S -methionine labelled protamine were dissolved in 100 μl . of 50 per cent aqueous pyridine and 100 μl . of pyridine-water-N-ethylmorpholine buffer (4:4:1) (NEMO). Phenylisothiocyanate (100 μl .) was added, mixed and deoxygenated by bubbling nitrogen through the solution. The tubes were sealed and incubated at 37° C for 3 h; they were then extracted three times with benzene and lyophilized. Cyclization was performed with anhydrous trifluoroacetic acid at 37° C for 1 h. The samples were lyophilized, redissolved in water and extracted three times with 5 volumes of ethyl acetate; the extracts were pooled, lyophilized, treated with 1 M HCl at 80° C for 15 min and re-lyophilized. Aliquots of the aqueous and ethyl acetate phases were counted in Bray's solution; an aliquot of the ethyl acetate extractable material was subjected to thin-layer chromatography on silica gel with the solvent heptane-n-butanol-formic acid (100:60:18) for 2 h. The gel was scraped off the plate at 0.5 cm intervals and counted in toluene scintillation fluid.

recovered in the ethyl acetate phase after one Edman degradation. This labelled PTH derivative co-chromatographed with a PTH-methionine marker on thin-layer chromatography (legend to Table 1). After dansylation of ^{35}S -methionine labelled protamine and separation of the acid hydrolysate by electrophoresis at pH 4.38 (ref. 19), up to 70 per cent of the label was recovered as dansyl- ^{35}S -methionine. The lack of solubility of protamine in the solvents used in these N-terminal methods probably explains the failure to recover closer to 100 per cent of the label.

Structure of the Methionine Peptide

When ^{35}S -methionine labelled protamine from cell incubations is digested with trypsin, a labelled basic peptide, A, is seen (Fig. 2) after high-voltage electrophoresis at pH 3.6 and autoradiography. A trace of a slightly basic peptide has been seen occasionally, but both peptides change mobility after acetylation, indicating that neither has a blocked N-terminus. As shown in Fig. 2, further digestion of peptide A with carboxypeptidase B yields a labelled peptide, B, which has the same electrophoretic mobility as the Met- ^{14}C -Pro marker (see legend to Fig. 2), and some intact peptide A remains. The electrophoretic behaviour of A and its carboxypeptidase B product, B, is consistent with the structures Met-Pro-Arg for A and Met-Pro for B. Deformylation of the N-Formyl- ^{14}C -Met-Pro-Arg marker caused it to streak during electrophoresis; ^{35}S -methionine-labelled protamine from a cell incubation was therefore formylated chemically in order to compare the N-formyl labelled peptides derived from it with N-formyl marker peptides.

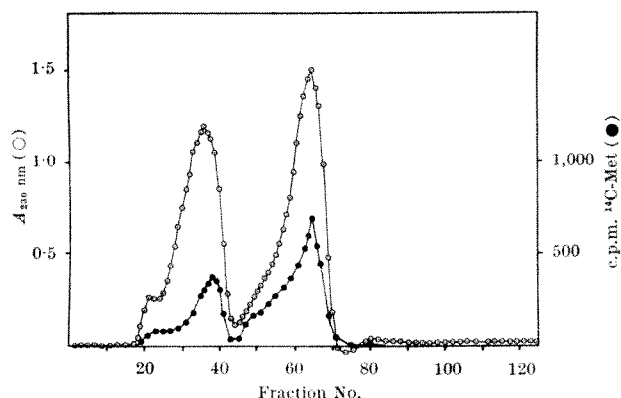


Fig. 1. A cell suspension was prepared from 25 g of testis at a late stage of development using Hanks's balanced salt solution; the cells were incubated with 1 $\mu\text{Ci/ml}$. of [^{14}C -methyl]-methionine (11 mCi/mmol) for 2 h at 20° C. The cells were washed and homogenized in saline-EDTA (0.075 M NaCl, 0.024 M ethylenediaminetetraacetic acid, pH 8) and nuclei sedimented at 3,000g for 10 min; the nuclear pellet was extracted twice with 0.2 M sulphuric acid and the acid-soluble proteins precipitated after addition of 4 volumes of ethanol at -20° C overnight. The precipitate was centrifuged, redissolved and applied to a 'Bio-Gel P-10' column (5 \times 45 cm) which was eluted with 0.2 M acetic acid; 12 ml. fractions were collected and 0.5 ml. of each was counted in Bray's scintillation fluid.

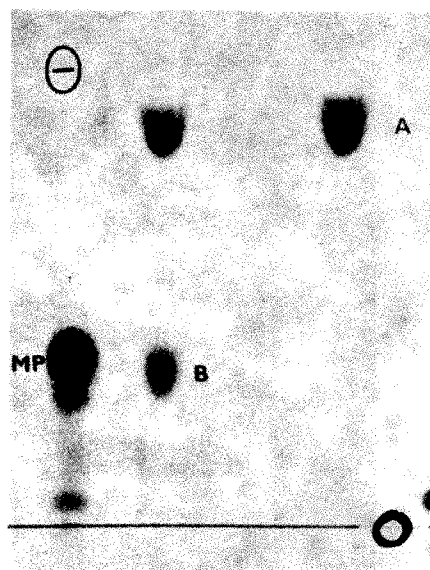


Fig. 2. Autoradiogram of digests of labelled protamine from a cell incubation. 20 g of hormonally induced trout testis was incubated in 40 ml. of Hanks's solution with 20 $\mu\text{Ci/ml}$. of ^{35}S -methionine (500 mCi/mmol) at 18° C for 3 h. Protamine was extracted, purified and a portion digested with porcine trypsin (1 μg enzyme/20 μg sample) at 37° C for 4 h yielded peptide A; further digestion with carboxypeptidase B (1 μg enzyme/ μg sample) at 37° C for 8 h yielded peptide B together with intact A. MP is the Met- ^{14}C -Pro marker. Electrophoresis was at pH 3.6 in pyridine-acetic acid-water (1:10:89) for 1 h at 40 V/cm. The electrophoretogram was exposed to Kodak 'Royal Blue' X-ray film for 6 days. Met- ^{14}C -Pro and its formylated derivative were synthesized by coupling *tert*-butoxycarbonylmethyl-N-hydroxysuccinimide ester to ^{14}C -proline²⁰; the blocking group was removed with anhydrous trifluoroacetic acid (37° C, 1 h) and was purified by high-voltage electrophoresis at pH 3.6. Met- ^{14}C -Pro was eluted from the paper and a portion was formylated by the mixed anhydride method of Sheehan and Yang²¹. Because protamine has a single N-terminal secondary amino group and no lysine, it is possible to couple amino-acids specifically to the N-terminus without blocking any groups on the protamine. Thus ^{14}C -methionine was formylated and coupled to N-hydroxysuccinimide using dicyclohexylcarbodiimide as the condensing agent; the active ester was then reacted with protamine in water-ethanol-dioxane (7:2:2) at room temperature overnight. The product was incubated at pH 10 and 37° C for 30 min to de-esterify any serine residues that might have reacted with the active ester. The N-formylmethionyl-protamine was then purified by chromatography on 'Sephadex G-10' and digested with trypsin (1:20) at 37° C for 4 h in 0.2 M ammonium bicarbonate, and a portion of this digest was deformylated with anhydrous 1 M HCl in methanol at 50° C for 1 h. Because the N-terminal sequence of protamine is Pro-Arg-Arg...¹¹, the above procedures yielded the tryptic peptide, N-formyl- ^{14}C -Met-Pro-Arg and its deformylated derivative, ^{14}C -Met-Pro-Arg.

Formylated ^{35}S -methionine labelled protamine was digested with trypsin or trypsin followed by carboxypeptidase B and the digests were electrophoresed at pH 3.6. Trypsin alone yielded a neutral or slightly basic peptide, A_r, which had the same mobility as the N-Formyl- ^{14}C -Met-Pro-Arg marker; this was confirmed by re-electrophoresis at pH 1.9. Trypsin plus carboxypeptidase B gave a labelled acidic peptide, B_r, which migrated together with the N-Formyl-Met- ^{14}C -Pro marker; this was confirmed by re-electrophoresis at pH 6.5 (Fig. 3a) and descending chromatography (Fig. 3b). Clearly therefore the methionine incorporated into protamine *in vivo* is present in the sequence, Met-Pro-Arg....

Role of Methionine

It was of interest to see if methionine is incorporated into the several components of protamine^{11,12} rather than a single minor component. Cells were incubated with ^{35}S -methionine and protamine was purified and chromatographed on a CM-cellulose column eluted with a linear gradient of lithium chloride¹². Fig. 4 shows six peaks of radioactivity extending across the absorbance profile of the carrier protamine. As mentioned previously, newly synthesized protamine is phosphorylated on its seryl residues; this modification affects the behaviour of protamine on CM-cellulose¹⁷. After digestion with *E. coli* alkaline phosphatase and re-chromatography on CM-cellulose, ^{35}S -methionine labelled protamine still exhibited

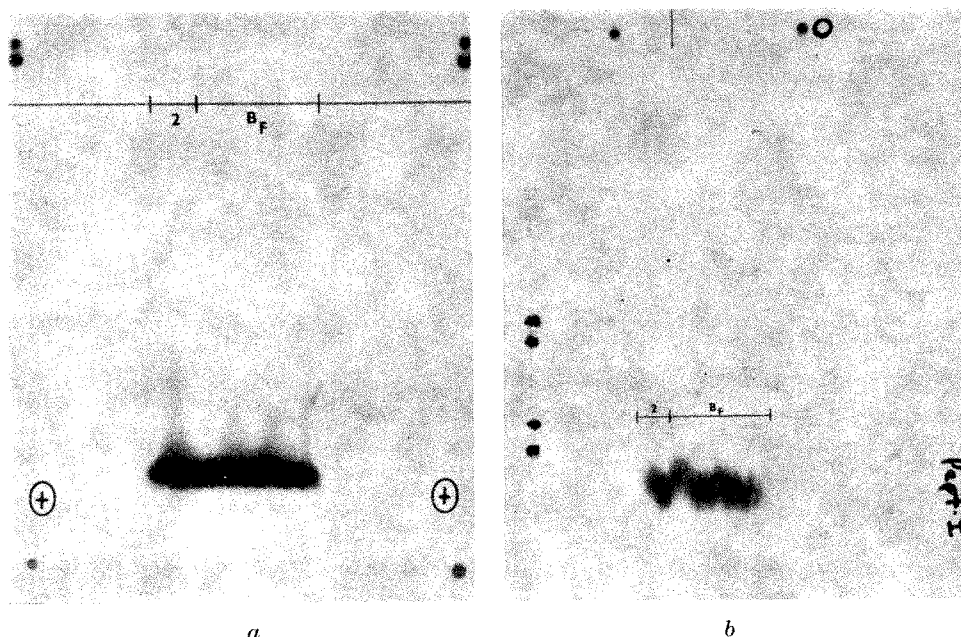


Fig. 3. *a*, Autoradiogram of re-electrophoresis of peptide B₁ from a trypsin digest of chemically formylated ³⁵S-methionine labelled protamine. N-Formyl-Met-¹⁴C-Pro marker was applied at 2 and the digest at B_F. Electrophoresis was at pH 6.5 in pyridine-acetic acid-water (10:0.4:89.6), for 1 h at 50 V/cm. The electrophoretogram was exposed to X-ray film for 7 days. *b*, Descending chromatography. Peptide B₁ and the N-formyl-Met-¹⁴C-Pro marker were cut out of the paper from which the autoradiogram in *a* was obtained, sewn into a new sheet of Whatman '3 MM' chromatography paper and subjected to descending chromatography in butanol-acetic acid-water-pyridine (15:3:12:10) for 16 h. The chromatogram was exposed to X-ray film for 5 days.

six peaks of label across the absorbance profile. Methionine must have been incorporated into several protamine components therefore and because tryptic digestion of whole protamine gave only Met-Pro-Arg, in every component the incorporation must have been at the N-terminal position.

If methionine has a role in initiation of protamine synthesis, its presence at the N-terminus should be transient as it is in bacterial systems. To test this, testis cell suspensions were pulsed with ³⁵S-methionine and ³H-arginine and then chased in fresh medium containing the unlabelled amino-acids. Fig. 5 reveals that incorporated arginine is stable during the course of the incubation whereas the methionine is chased out, at least partially. In a second experiment the ratio of ³⁵S-methionine to ³H-arginine in protamine components was examined following separation of newly synthesized protamine on starch-urea-aluminium lactate gels¹⁶ and counting of the radioactivity in the sliced gel. The methionine: arginine ratio approached zero 60 min after the chase began. These results show that the N-terminal methionine is removed after chain completion, but they do not eliminate the possibility that some removal occurs before chain completion.

Extracts of trout testis contain an enzyme activity that can cleave the Met-Pro peptide bond; Table 2 demonstrates the presence of this enzyme activity in nucleoplasm, high-speed supernatant and in the ribosomal wash. The testis extracts are also active against ³⁵S-methionine labelled protamine, but the presence of other proteases in the crude preparations which fragment protamine requires that the Met-Pro hydrolysing activity be purified before its specificity and other properties can be tested. It is possible, however, that the activity detected above is due to a specific methionyl-aminopeptidase responsible for removing

methionine from the N-terminus of protamine and perhaps other proteins.

Inhibitors

Methionine incorporation into protamine was found to be very sensitive to inhibition by cycloheximide with 50 per cent inhibition at less than 10⁻⁷ M. This extreme sensitivity to cycloheximide is characteristic of eukaryotic cytoplasmic protein synthesis and has been described previously for arginine incorporation into protamine¹⁴. Cycloheximide inhibits both chain initiation and elongation²², so that the great sensitivity of methionine incorporation to this inhibitor rules out a mechanism involving N-terminal addition of amino-acids as described for *E.*

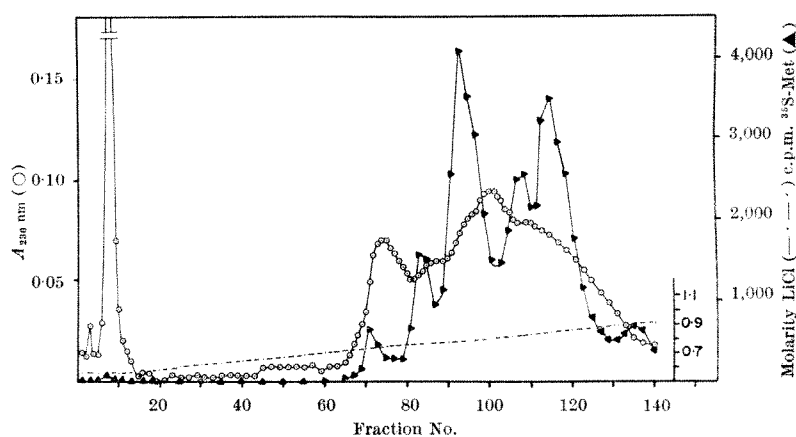


Fig. 4. Elution profile of ³⁵S-methionine labelled protamine on a CM-cellulose column eluted with a linear gradient of lithium chloride¹⁴. A cell suspension was prepared from 20 g of rainbow trout testis in which spermatogenesis was artificially induced with injections of pituitary extract from *S. oncorhynchus*²³. The cells were incubated with ³⁵S-methionine (500 mCi/mmol) at 20 μ Ci/ml. for 3 h at 18 $^{\circ}$ C; the cells were washed, homogenized in 50 mM Tris-HCl, 5 mM magnesium acetate, 25 mM KCl, 0.25 M sucrose (pH 7.6) (TMKS) and nuclei were pelleted at 3,000g for 10 min. The nuclei were extracted twice with cold 0.2 M HCl, the extract was neutralized with LiOH and applied to a CM-cellulose column (1.5 \times 35 cm) and eluted with a LiCl gradient: the protamine region was pooled, desalted, lyophilized and reappplied to a second CM-cellulose column (2.5 \times 26 cm) and eluted with a linear gradient generated from 600 ml. each of 0.6 and 1.3 M LiCl with the result shown. 8 ml. fractions were collected and 0.3 ml. of each was counted in Bray's scintillation fluid; salt concentration was measured using a conductivity meter and reference to standard solutions of LiCl.

Table 2. Met-¹⁴C-Pro HYDROLYSIS

Cell fraction	Percentage hydrolysed in 45 min at 16° C	Relative activity*	Percentage total
(1) High-speed supernatant	73.9	100	57
(2) Nucleoplasm	65.8	50	28
(3) Ribosome wash	53.4	27	15

* Corrected for volume of extract per g of testis.

Enzyme extracts were prepared by homogenizing October-stage trout testis in TMKS containing 10^{-4} M dithiothreitol; nuclei were pelleted at 1,500g for 10 min, mitochondria at 20,000g for 30 min and ribosomes at 25,000 r.p.m. in the Spinco 'SW-27' rotor for 4 h. Nuclei and mitochondria were lysed in TMK and centrifuged at 20,000g for 20 min; the supernatants were saved. Ribosomes were washed with 1 M NH_4Cl in TMK overnight; the suspension was centrifuged at 26,000 r.p.m. in the 'SW-27' rotor for 6 h and the supernatant saved. All extracts were precipitated at 90 per cent saturation with $(\text{NH}_4)_2\text{SO}_4$, the precipitate was redissolved in TMK (pH 7.0) and dialysed against three changes of TMK; material precipitating during dialysis was removed by centrifugation. 50 μl aliquots of the extracts were incubated with 50 μl of Met-¹⁴C-Pro in TMK for 45 min at 16° C and the reaction was stopped by chilling and adding to 10 μl 1 M KOH. The samples were electrophoresed at pH 3.6 and 40 V/cm for 45 min; products were located by autoradiography; corresponding areas were cut out and counted in toluene scintillation fluid.

coli^{23,24} and rat liver²⁵. Insensitivity of protamine synthesis to all but high levels of chloramphenicol¹⁴, together with work on the intracellular location of protamine synthesis^{12,26}, seems to eliminate a mitochondrial site of synthesis.

Nascent Peptides

Testis cells were incubated with ³⁵S-methionine for 20 min at 15° C, and ribosomes were prepared and fractionated by centrifugation on a linear gradient of sucrose, with the result illustrated in Fig. 6. Methionine is incorporated generally across the polysome region with the specific activity (c.p.m. ³⁵S-methionine per A_{254}) increasing with the size of the polysome. Because protamine synthesis has been shown to occur on disomes¹², the monosome and disome peaks in the gradient above were pooled and the ribosomes pelleted by centrifugation; ribosome-bound nascent peptides were released, digested with trypsin and a portion was further digested with carboxypeptidase B; the digests were electrophoresed at pH 3.6 with marker peptides. The label was present in the neutral region and in peptides that had the same

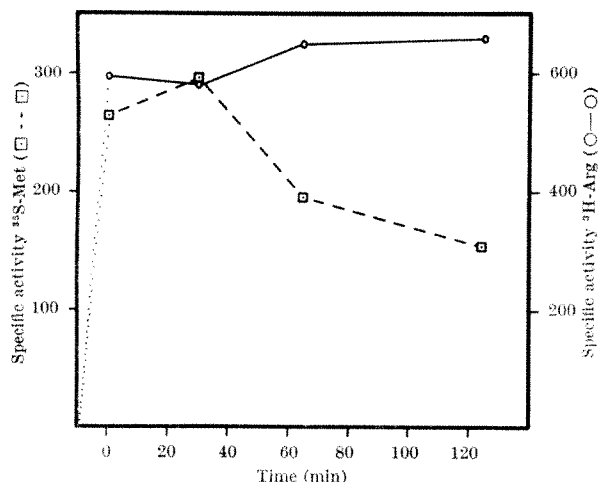


Fig. 5. Pulse-chase experiment. A cell suspension was prepared from 15 g of naturally maturing trout testis with 10 ml. of Hanks's. The suspension was divided into two: 4 $\mu\text{Ci}/\text{ml}$. of ³⁵S-methionine (573 mCi/mole) was added to one half and 4 $\mu\text{Ci}/\text{ml}$. of ³H-arginine (1.2 Ci/mole) to the other. Incubation was at 18° C for 10 min; the cells were chilled, washed and resuspended in 20 ml. of Hanks's medium which was 1 mM in unlabelled methionine and arginine, and incubation was continued at 18° C. 5 ml. aliquots were removed starting immediately after resuspending and were frozen in a bath of solid CO_2 ; these were homogenized in saline-EDTA and nuclei were pelleted at 3,000g for 10 min and extracted twice with 0.2 M HCl. The acid extracts were neutralized with LiOH and applied to CM-cellulose columns (1.5 × 5 cm), eluted with 0.75 M LiCl, washed with water and re-eluted with 0.2 M HCl. The acid eluate containing the protamine was lyophilized and then further purified by chromatography on a 'Bio-Gel P-10' column (2.6 × 15 cm) in 0.2 M acetic acid. The protamine peaks were lyophilized and redissolved in distilled water, absorbance at 230 nm was measured, and aliquots were counted in Bray's solution. Specific activity was calculated as c.p.m. of ³⁵S-methionine per A_{230} unit of protamine.

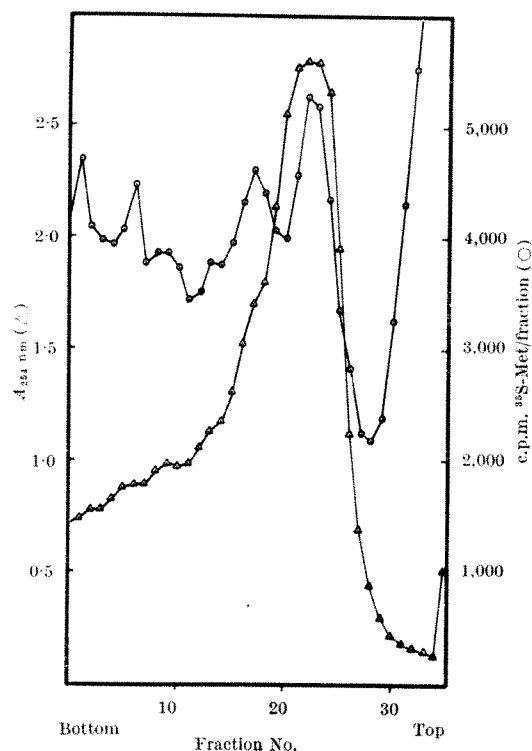


Fig. 6. Ribosomes from ³⁵S-methionine incubation fractionated on a sucrose gradient. A cell suspension from 12 g of induced trout testis was incubated at 15° C for 20 min with ³⁵S-methionine (690 mCi/mole) present at 20 $\mu\text{Ci}/\text{ml}$.; at 15 min, cycloheximide was added to a final concentration of 2×10^{-4} M. The cells were washed and then homogenized in TMKS; the homogenate was centrifuged at 15,000g and then homogenized in TMK; 20 per cent in TMK (TMKS minus sucrose) was added to the supernatant to give a final concentration of 0.5 per cent and centrifuged at 15,000g for 20 min. The supernatant was layered over 2 ml. of 30 per cent sucrose in 13 ml. tubes and centrifuged at 60,000 r.p.m. in the Spinco 'L2-45' rotor for 2.5 h. The supernatant was aspirated and the pellet washed gently with TMK; one-third of the ribosomes were resuspended in 1 ml. TMK and layered over a 35 ml. 10–30 per cent linear gradient of sucrose in TMK generated by a Beckman density-gradient former ('DGF-IM-3'). Centrifugation was at 25,000 r.p.m. in the Spinco 'SW-27' rotor for 3.5 h at 4° C; the centrifuge was stopped without the brake, the tubes punctured and 1 ml. fractions collected. The absorbance at 254 nm was measured and the radioactivity in 200 μl aliquots counted in Bray's solution.

mobilities as Met-Pro and Met-Pro-Arg markers; these labelled areas were cut out, sewn to a new sheet of paper and re-electrophoresed at pH 1.9 with markers. This procedure showed that the neutral material is free methionine and confirmed that the other two peptides are Met-Pro and Met-Pro-Arg; there was no evidence of labelled material corresponding to formylated marker peptides. This absence of formylated nascent peptides suggests that either the methionine is never formylated or the formyl group must be removed early, rapidly and completely on the ribosome.

¹⁴C-Formate is incorporated into protamine by testis cells *in vitro*. Protamine labelled in this manner was hydrolysed with 6 M HCl at 110° C for 16 h; the label was not volatile and was found to have the electrophoretic mobility of serine at pH 1.9. Presumably this is because of the presence in trout testis of a hydroxymethyl transferase which can synthesize serine from glycine and N⁵,10-methylene tetrahydrofolate, the methylene moiety being derived from formate. Attempts to find ¹⁴C-formate or ³⁵S-methionine-labelled formylated nascent peptides either on the ribosomes or in the high-speed supernatant after puromycin release were unsuccessful.

The transient incorporation of methionine at the N-terminus of several protamine components suggests that it has a role in the initiation of biosynthesis of this protein. The ease with which this incorporation can be detected may be related to the unusual properties of protamine. Rich, Eikenberry and Malkin^{27,28} have

demonstrated a shielding effect of the ribosome on nascent proteins; ribosome-bound nascent chains 30–35 residues long were found to be protected from external proteolytic attack. Protamine, 31–32 residues long, may therefore be protected from attack by a putative methionine-removing enzyme until the chain is completed and released into the cytoplasm. Other contributory circumstances may be the difficulty of cleavage of the Met-Pro peptide bond; in general, X-Pro bonds are resistant to many proteases with the possible exception of imidodipeptidase³⁰; but this enzyme is specific only for dipeptides. Also, as shown by Ling and Dixon²⁶, at the stage of differentiation at which protamine synthesis is maximal, there has been a considerable reduction of the spermatid cytoplasm and ribosome content. The levels of cytoplasmic enzymes, including the methionine-removing enzyme, may therefore become limiting at this stage.

The involvement of methionine in the initiation of protein synthesis in eukaryotic cells is not yet firmly established although the presence of a formylatable species of Met-tRNA with the properties of an initiator tRNA in mouse ascites cells^{31,32} is certainly consistent with such a role. Our observations of methionine involvement in the synthesis of the very unusual, sperm-specific polypeptide, protamine, suggest that such a mechanism may be of widespread importance in eukaryotes, and in the special circumstances of protamine biosynthesis, where removal of the N-terminal methionyl residue may become limiting, the transient incorporation of this amino-acid is readily observed.

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Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4

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Using an improved method of gel electrophoresis, many hitherto unknown proteins have been found in bacteriophage T4 and some of these have been identified with specific gene products. Four major components of the head are cleaved during the process of assembly, apparently after the precursor proteins have assembled into some large intermediate structure.

BACTERIOPHAGES of the T-even type are complex structures containing many different proteins and specified by many genes. Using an improved technique of electrophoretic separation I have found that the phage particle contains at least twenty-eight components, eleven of which are in the head. In the course of identifying the genes specifying these proteins I discovered that four major components of the head, the product of gene 22, 23, 24 and a protein called IP of unknown genetic origin are cleaved during the process of assembly. The head of bacteriophage T4 is therefore no longer a self-assembly system in the narrow sense, because the bonding properties of the various components become altered during the assembly process.

The product of gene 23 is the principal protein component of the head of bacteriophage T4 (refs. 1–3). Two minor components of unknown genetic origin have also been found in capsids^{2,3}. Besides the product of gene 23, the products of genes 20, 21, 22, 24, 31, 40 and 66 are required to determine the size and shape of the head-shell (refs. 4 and 5 and unpublished work of F. A. Eiserling,

E. P. Geiduschek, R. H. Epstein and E. J. Metter). To several of these genes shape-specifying functions have been tentatively assigned⁵. Gene 22 is associated with the diameter selecting (initiation) process of head formation, gene 66 with the elongation of the particle and genes 20 and 40 with the formation of the hemispherical cap. Gene 31 somehow modifies or activates the major subunit for ordered assembly⁶. Ten more proteins, the products of genes 2, 4, 13, 14, 16, 17, 49, 50, 64 and 65, are thought to control later steps in head formation⁷.

Structural Components of the Phage

Many phage proteins can be separated with our improved method of disk-electrophoresis in sodium dodecyl sulphate (SDS). This system, to be described in detail elsewhere (U. K. L. and J. V. Maizel), combines the high resolution power of disk-electrophoresis⁸ with the capability of SDS to break down proteins into their individual polypeptide chains⁹. The proteins are also separated according to their molecular weight as was first reported for a continuous system¹⁰. All the proteins the genetic

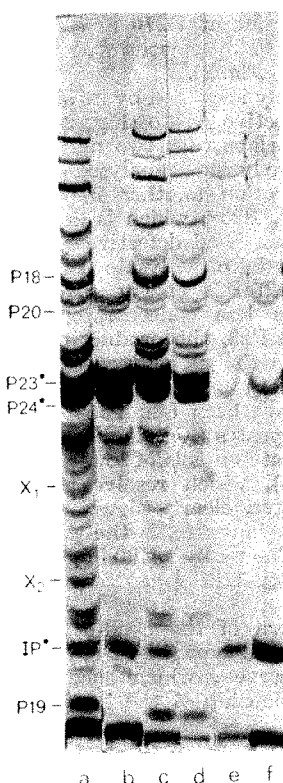


Fig. 1. Autoradiogram of ^{14}C -labelled T4 phage proteins separated in acrylamide gels. ^{14}C -amino-acid-labelled preparations were analysed in 10 per cent acrylamide gels containing SDS. a, Wild type lysate; b, purified heads; c, purified phage particles; d, purified "ghosted" phage particles; e, supernatant of "ghosted" phage particles; f, "early labelled" phage particles. The prefix P is used to designate the protein of a particular gene: for example, P20 stands for the product of gene 20; the asterisk indicates that this protein is derived from a large precursor protein and has become modified during head assembly.

^{14}C -labelled lysates. Ten ml. cultures of *Escherichia coli* B^h (the restrictive host for phage carrying amber mutations) in 'M9' medium¹⁹ grown at 37°C to 2×10^8 cells/ml. were infected with the various phages at a multiplicity of five and superinfected at 8 min with the same phage and the same multiplicity to ensure lysis inhibition. Two μCi of ^{14}C -amino-acid mixture ('CFB 104', Radiochemical Centre, Amersham) with a specific activity of 45 mCi/m atom was added to each culture 13 min after the first infection. Three ml. of 3 per cent 'casamino-acids' mixture (Difco) was added to each sample at 30 min, and the infected cells were concentrated by low speed centrifugation 35 min following infection. The pellets were drained and directly resuspended in "final sample" buffer (see gel electrophoresis).

^{14}C -labelled phage and head particles. Ten ml. cultures were grown and infected as described above (^{14}C -labelled lysates). The double mutant (B255-E18) in genes 10 and 18 was used for production of tailless heads. Ten μCi of the ^{14}C -amino-acid mixture was added 13 min after the first infection to each culture. The infected cells were concentrated by a low speed centrifugation 35 min after infection and the pellet was resuspended in 1 ml. neutral phosphate buffer containing 10^{-3} M MgSO_4 , 20 μg deoxyribonuclease and a drop of chloroform. The pellet was resuspended by repeated pipetting, and incubated for 15–30 min at room temperature before layering on a CsCl step gradient²⁰. The latter was prepared in tubes for a Spinco 'SW50' rotor, with 0.8 ml. layers, and the following densities starting at the bottom of the tube: 1.55, 1.46, 1.38 and 1.29 g/cm³. Furthermore, a 10 per cent sucrose solution (in neutral phosphate buffer and 10^{-3} M MgSO_4) was layered on the last CsCl step to prevent precipitation of soluble proteins at the CsCl interface. Centrifugation was for 1 h at 40,000 r.p.m. The phage and heads, which form a sharp visible band two-thirds down the tube, were collected through the bottom of the tube and dialysed against water. The band containing the heads was always viscous, indicating that the heads lost their DNA in CsCl although the DNA was still confined within the band. Occasionally the heads lost their DNA before centrifugation particularly in more concentrated lysates, which had to be treated with deoxyribonuclease for a much longer time. "Early labelled" phages were prepared identically, but the label was added 1 min and chased 6 min after infection by the addition of 3 ml. of 3 per cent 'casamino-acids'.

"Ghosted" phage particles and supernatant of "ghosted" phage particles. Crystalline NaCl was added to ^{14}C -labelled purified phage particles in water to a final concentration of 5 M. The preparation was then repeatedly frozen in a solid CO_2 -acetone bath and thawed in warm water. This procedure releases the DNA and its internal proteins from the phage head. The sample was dialysed against neutral phosphate buffer containing 10^{-3} M MgSO_4 and the DNA digested by the addition of a small amount of crystalline deoxyribonuclease. The ghost was finally separated from the soluble proteins (internal proteins) by centrifugation and layered on a step gradient identical to that already described. Centrifugation was at 35,000 r.p.m. for 1 h. The top of the gradient containing the internal proteins was collected with a pipette and the ghosts, which band at an approximate density of 1.3 g/cm³, were collected through the bottom of the tube. SDS at a final concentration of 2 per cent was added to both samples and the samples were dialysed against water containing 2 per cent SDS.

Gel electrophoresis. Gels containing 3 per cent (stacking gel), 8.0 per cent or 10 per cent acrylamide were prepared from a stock solution of 30 per cent by weight of acrylamide and 0.8 per cent by weight of N,N'-bis-methylene acrylamide. The final concentrations in the separation gel were as follows: 0.375 M Tris-HCl (pH 8.8) and 0.1 per cent SDS. The gels were polymerized chemically by the addition of 0.025 per cent by volume of tetramethylethylenediamine (TEMED) and ammonium persulphate. Ten cm gels were prepared in glass tubes of a total length of 15 cm and with an inside diameter of 6 mm. The stacking gels of 3 per cent acrylamide and a length of 1 cm contained 0.125 M Tris-HCl (pH 6.8) and 0.1 per cent SDS and were polymerized chemically in the same way as for the separating gel. The electrode buffer (pH 8.3) contained 0.025 M Tris and 0.192 M glycine and 0.1 per cent SDS. The samples (0.2–0.3 ml.) contained the final concentrations ("final sample buffer"): 0.0625 M Tris-HCl (pH 6.8), 2 per cent SDS, 10 per cent glycerol, 5 per cent 2-mercaptoethanol and 0.001 per cent bromophenol blue as the dye. The proteins were completely dissociated by immersing the samples for 1.5 min in boiling water²¹. Electrophoresis was carried out with a current of 3 mA per gel until the bromophenol blue marker reached the bottom of the gel (about 7 h). The proteins were fixed in the gel with 50 per cent trichloroacetic acid (TCA) overnight, stained for 1 h at 37°C with a 0.1 per cent Coomassie brilliant blue solution made up freshly in 50 per cent TCA. The gels were diffusion-destained by repeated washing in 7 per cent acetic acid. Autoradiograms of gels were prepared by a modified version (U. K. L. and J. V. Maizel, unpublished) of Fairbanks *et al.*²² (autoradiograms are shown in Figs. 1–7).

origin of which was determined are labelled in Fig. 1, and their molecular weights are listed in Table 1. At least 28 bands can be distinguished in the autoradiogram of radioactively labelled, purified phage particles (Fig. 1c). The 28 proteins found in dissociated phage particles do not include proteins with molecular weights less than about 15,000. Those proteins are not sieved in gels of 10 per cent acrylamide (unpublished results of U. K. L. and J. V. Maizel) and migrate with the marker dye. In gels of higher acrylamide concentration another three low molecular weight proteins have been separated (results not shown). The largest protein in the phage has an approximate molecular weight of at least 120,000 and no label stays at the top of the gel, indicating complete dissociation of the particles by the method used.

Eleven of these 28 proteins are found in the purified head preparation (Fig. 1b). The remaining 17 proteins absent from the head but present in the whole phage pattern are presumably structural proteins of the tail and tail fibres. The complete absence of these proteins from the head gel pattern indicates the high degree of purity of the preparation. The classification of the proteins into tail and head components may not be valid for proteins making up the head to tail junction.

Only two minor proteins besides the major components P23 were found by others^{2,3} in phage capsids purified in the same way but fractionated in urea gels. The larger number of proteins found in SDS gels is to be expected, for at least 46 genes are known to affect T4 morphogenesis^{4,7} although the proteins of these genes may not all be incorporated into the particle.

The gel pattern of a total lysate of wild type infected cells radioactively labelled at late times is presented in Fig. 1a. Note that most of the resolved proteins that

are synthesized late in infection are structural phage components. A few rather intense bands (X_1 and X_2), however, are completely missing in the phage particles, also demonstrating that the separation of the phage particles from the soluble proteins is complete.

When phages are subjected to osmotic shock a number of proteins are released¹¹. The principal one is IP*, an internal protein (Fig. 1). It is present in complete phage particles, is extracted from the phage particles by freezing and thawing in high salt concentrations and is quantitatively recovered in the supernatant. The supernatant fraction also contains many minor components which are found both in phages as well as phage ghosts, indicating that the

Table 1. MOLECULAR WEIGHTS OF PHAGE PROTEINS DETERMINED BY COMPARING THEIR MOBILITY IN SDS GELS WITH THOSE OF MARKER PROTEINS WITH KNOWN MOLECULAR WEIGHTS^{10,22}

Gene product	Observed value in SDS gels	Published value
P18	69,000	50,000 ⁸
P20	63,000	
P23	56,000	
P23*	46,500	46,000 ²¹
P24	45,000	
P24*	43,500	
P22	31,000	
IP	23,500	
IP*	21,000	
P19	18,000	

The following marker proteins were used: serum albumin (68,000), γ -globulin, heavy chain (50,000), ovalbumin (43,000), γ -globulin, light chain (23,500) and TMV (17,000), to calibrate 10 per cent acrylamide gels. In this improved gel system the distances of migration of the various marker proteins relative to the distance of migration of bromophenol blue are also a linear function of the logarithm of the molecular weight of the marker proteins, as has been described^{10,22}. Radioactively labelled phage proteins were mixed with unlabelled marker proteins before electrophoresis; the distance of migration of the phage proteins was determined from the autoradiogram and those of the marker proteins from the stained gel. It is assumed that the phage proteins also separate in SDS gels solely according to their molecular weight.

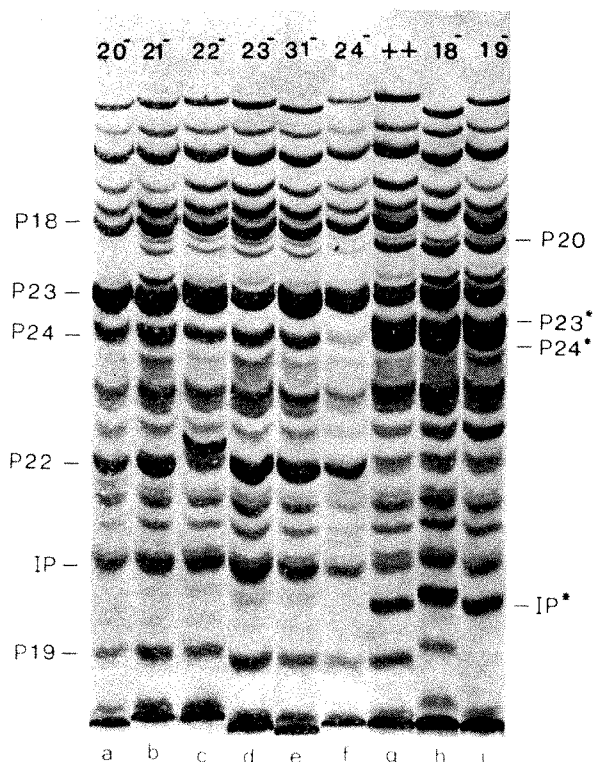


Fig. 2. Identification of gene products on 10 per cent acrylamide gels. The lysates were prepared as described in Fig. 1 and analysed on 10 per cent acrylamide gels. *a*, Gene 20-defective lysate, mutant N50; *b*, gene 21-defective lysate, mutant N90; *c*, gene 22-defective lysate, mutant B270; *d*, gene 23-defective lysate, mutant H11; *e*, gene 31-defective lysate, mutant N54; *f*, gene 24-defective lysate, mutant N65; *g*, lysate from wild type infected cell; *h*, gene 18-defective lysate, mutant E18; *i*, gene 19-defective lysate, mutant E1137. Mutants N90 (gene 21), E18 (gene 18) and E1137 (gene 19) carried second mutations in gene 10 (mutant B255). The following amber fragments may be detected. A rather intense band just below P23 is consistently seen in 18-defective lysates, and is presumed to be the amber fragment of mutant E18 in gene 18. Furthermore, the gel pattern of defective lysates of all double mutants in genes 18-10, 21-10, and 19-10 possess a band just above P23 which is probably the amber fragment of mutant B255 in gene 10. It is striking that the amber fragment of mutant B270 gene 22 behaves anomalously in the gel: it migrates more slowly than the wild type product, although SDS gels are known to separate on the basis of molecular weights. This anomalous behaviour of certain proteins will be discussed elsewhere (U. K. L. and J. V. Maizel).

separation of the released proteins and the ghosted particles by centrifugation was not complete. Some of these proteins, however, are extracted into the supernatant quantitatively by the freezing and thawing procedure.

Only the protein IP*, and perhaps some low molecular weight proteins which migrate with the marker dye, are structural phage components synthesized at early times (early proteins). This can be seen in the gel pattern of purified phage labelled at early times only (Fig. 1*f*). IP* is also labelled at late times. All the other structural phage components are synthesized only late in infection. Some of the principal late proteins show up on the autoradiogram, probably because of residual incorporation of radioactive amino-acids following the chase with unlabelled amino-acids.

Identification of Gene Products

The products of genes 18, 19, 20, 22, 23 and 24 were identified by comparing the gel pattern of extracts of cells infected with wild type phage with those infected with amber mutants in various genes. The identification of the tail and tail fibre proteins will be described later (J. King and U. K. L.). Amber mutations produce only fragments of the protein chain of the mutant gene on infection of restrictive bacteria¹. These fragments migrate differently in the gel from the complete proteins. The molecular weights of the proteins identified are listed in Table 1. Fig. 2 is the autoradiogram from dried and sliced gels for

various mutants. The product of gene 20 is identified by its absence in the gel pattern of a 20-defective* lysate (Figs. 2 and 3*a*), and the product of gene 22 by its absence in the gel pattern of a 22-defective lysate (Figs. 2 and 3*c*). Note the amber fragment of mutant B270 in gene 22. This fragment was also identified by Hosoda and Levinthal¹³ in urea gels (see legend to Fig. 2).

The product of gene 23 is easily identified by its absence in the gel pattern of a 23-defective lysate (Fig. 2*d*). It can also be seen that P23 overlaps with two minor tail components. If the 23-defective lysate is analysed on gels of lower acrylamide concentration another important observation is made. A band, P23*, which is detected in variable amounts in the other head defective lysates, is completely missing in the 23-defective lysate (Fig. 3*d*). This band, P23*, overlaps with P24 in Fig. 2, but is better separated from P24 in less concentrated gels (Fig. 3). As with the product P23, the product of P24 was identified by its absence in the gel pattern of a 24-defective lysate (Figs. 2*f* and 3*e*).

So far, no missing bands have been found in the gel patterns of 31 or 21-defective lysates (Fig. 2*b* and *e*), but analysis of the 21-defective lysate on gels of lower acrylamide concentration (Fig. 3*b*), which resolves higher molecular weight proteins better, clearly shows that a band is missing. This protein, however, is the product of gene 10, a baseplate gene. The mutant N90 in gene 21 in fact carries a second mutation in gene 10 (mutant B255).

In comparing the gel pattern of the head-defective lysates (Fig. 2*a-f*) with that of wild type (Fig. 2*g*), further important differences are observed, which shed light on the precursor-product relationship of the head components.

The principal fraction of the gene 23 product has a molecular weight of 56,000 in all the head-defective lysates, but in wild type or tail-defective lysates it appears at the position of P23*, with a molecular weight of 46,500. Small but significant amounts of P23* are also observed in head-defective lysates (Fig. 3*a-f*). In lysates prepared identically about 20 per cent of the total P23 is converted to P23* in the 20-defective lysate, 10 per cent in 21 and 2-3 per cent in 22, 24 and 31-defective lysates, as determined from densitometer tracing of the autoradiographs.

The bands P22 and IP are both absent or considerably less intense in the wild type gel pattern (each overlaps with two other proteins). A new band, IP*, is seen at the bottom of the gel, which is completely missing in the head defective lysates (Fig. 2*a-f*).

The band P24, which is found in all head-defective lysates is missing in the wild type pattern, but a new band, P24*, which migrates slightly faster is observed. This is difficult to visualize in Fig. 2, but will become evident in Fig. 6.

Also included in Fig. 2 is the gel pattern of two tail defective lysates. The product of gene 18 (mol. wt 69,000), the principal protein of the tail sheath¹³, is identified by its absence in an 18-defective lysate (Fig. 2*h*) and the product of gene 19 (mol. wt 18,000) by its absence in a 19-defective lysate (Fig. 2*i*). P19 is thought to be the chief component of the tail tube¹⁴. This demonstrates that the differences in the head proteins are not related to tail attachment, for the gel patterns of the tail-defective lysates are identical with that of wild type.

Evidence will be presented that the proteins P23, P22, P24 and IP are cleaved in wild type infected cells and are precursors to proteins P23*, P24* and IP* found in the final head structure. (The cleavage product of P22 was not detected.) This precursor-product conversion is strongly inhibited by mutations in genes 20, 21, 22, 23, 24 and 31. Two important conclusions can be drawn: (a) the aberrant head-related structures—single and

* Henceforth, lysates of cells infected at restrictive conditions with amber mutants in various genes will be referred to as, for instance, a "21-defective lysate", where the amber mutant used was in gene 21.

multi-layered polyheads^{4,5,15}, τ -particles^{4,5,16} and lumps⁶ known to be produced in these mutant infected cells—chiefly consist of the precursor protein P23; (b) because P22 is not cleaved in these mutant infected cells, but is required for polyhead and τ -particle formation, as has been established by genetic means⁵, it is strongly suggested that P22 is incorporated into these structures as such.

Kinetics of the Cleavage Reactions

The following experiments were designed to study the precursor relationship of the proteins P23, P24 and IP with P23*, P24* and IP*, respectively. In this experiment the infected cells were pulse-labelled with radioactive amino-acids for a short period (1 min) and the modification of the various proteins was then followed by analysis of the samples taken at intervals in SDS gels. The results of autoradiography of the dried gels are presented in Fig. 4.

Cleavage of P23. It is readily seen in Fig. 4 that most of the 23 protein is at the position of P23 immediately following the pulse of the radioactive amino-acids. It then rapidly disappears, and a new band, P23*, appears simultaneously. That P23 is cleaved and gives rise to P23* is suggested by the fact that both are principal components and this is reinforced by the absence of P23 and P23* from the gel patterns of a 23 defective lysate (Fig. 3d). The kinetics of the cleavage reaction P23→P23* are plotted in Fig. 5a. Cleavage is very rapid: about 50 per cent of the precursor is cleaved within the first 2 min following chase of the label. P23* appears at about the same rate, in a satisfactorily correlated way. The total labelled protein in P23 and P23* is also plotted in Fig. 5a. The total label increases during the first minute, which reflects the completion time of the chase of the labelled amino-acid, but finally the total falls off by 25–30 per cent. This final decrease of the total labelled protein can be nicely explained, for a cleavage from about 56,000 to 46,500 corresponds to a loss of about 20 per cent by weight of protein.

Cleavage of P22 and IP. In the pulse-chase experiment of Fig. 4, two other protein bands, P22 and IP, disappear with time. (The disappearance of P22 in wild type infected cells

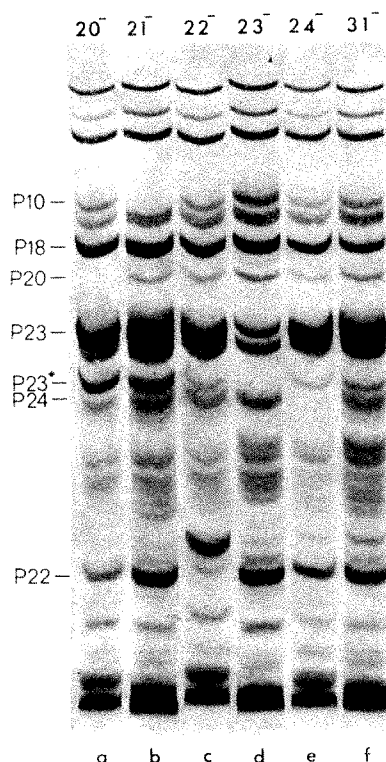


Fig. 3. Identification of gene products on 8 per cent acrylamide gels. ¹⁴C-labelled lysates were prepared as described (Fig. 1) and analysed on 8 per cent acrylamide gels. a, Gene 20-defective lysate, mutant N50; b, gene 21-defective lysate, mutant N90; c, gene 22-defective lysate, mutant B270; d, gene 23-defective lysate, mutant H11; e, gene 24-defective lysate, mutant N65; f, gene 31-defective lysate, mutant N54.

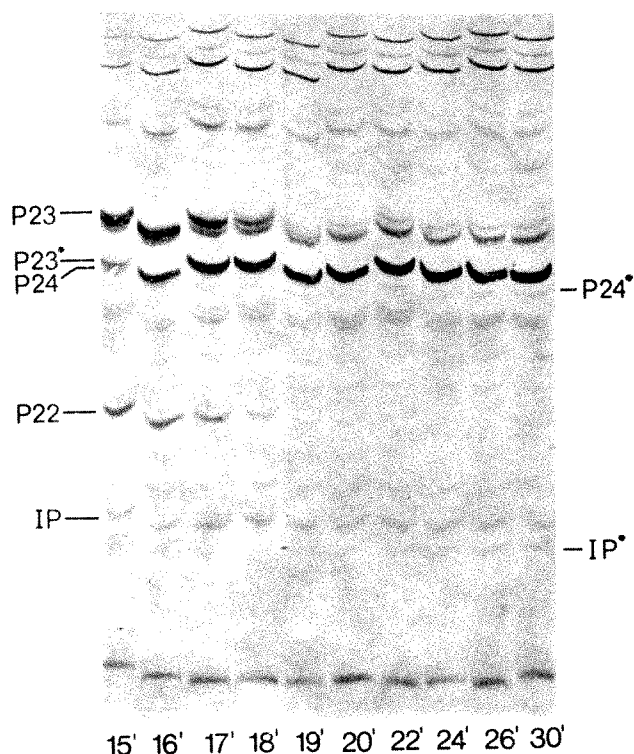


Fig. 4. Cleavage of products of genes 22, 23, 24 and protein IP (10 per cent acrylamide gels). A 10 ml. culture grown at 37° C was infected with a double mutant defective in genes 10 and 18 (mutant B255 and E18) as described (Fig. 1). The radioactive amino-acid mixture (10 μ Ci) was added 14 min after the first infection, and chased 1 min later with an excess of unlabelled amino-acids (final concentration 1 per cent). The chase of the label was verified by measuring the counts in the total TCA precipitable proteins. One ml. samples were prepared at intervals after the chase and immediately frozen in a solid CO₂-acetone bath. SDS was added after thawing to a final concentration of 2 per cent and the samples were carefully dialysed into 2 per cent SDS in water. The samples were finally mixed with an equal volume of twice concentrated "final sample buffer" and boiled for 1 min before electrophoresis. The sampling time is indicated at the bottom of the gels. All the preliminary experiments were done with wild type phage, but this experiment was performed with the double mutant in genes 10 and 18 in view of plans for future experiments.

has also been observed by M. Showe, personal communication.) A new band, IP*, appears at the bottom of the gel pattern. The kinetics of these cleavage reactions are plotted in Fig. 5b. P22 disappears with approximately the same initial rate as P23: about 50 per cent is cleaved 2–3 min following chase of the labelled amino-acids. I have not found a band in the gel pattern which may be derived from P22. Hosoda and Levinthal¹² reported indirect evidence that P22 is a structural phage component, but they considered the possibility that P22 might become altered during head formation.

Evidence for the precursor-product conversion IP→IP* is provided by the observation that the disappearance of IP and the appearance of IP* is coordinated in time (Fig. 5b). Furthermore, the total label lost at the IP position is recovered in the IP* band. The reaction is slower than that of P23 and P22. Only about 50 per cent of the total label at the IP position disappears. This could be explained either by another protein band overlapping with the IP band or synthesis in excess.

IP* cannot arise from P22. IP* must be derived from a precursor which is synthesized early, for I have shown (Fig. 1) that IP* is strongly labelled in the "early labelled" phage preparation. P22, however, is reported to be synthesized at late times only¹², which I have confirmed. The precursor conversion of IP→IP* has also been observed in a pulse-chase experiment in which the label was added between 4 and 5 min following infection, thus labelling only early proteins (results not shown). The band IP was easily recognized in these gels and the disappearance of IP and the appearance of IP* were again correlated in time. This quantitative agreement and the absence of other unaccounted changing bands in the gel pattern support the argument for the IP→IP* relationship. Of course, a final proof awaits chemical analysis.

It was also observed that, although the pulse was performed between 4 and 5 min after infection, cleavage of IP starts only

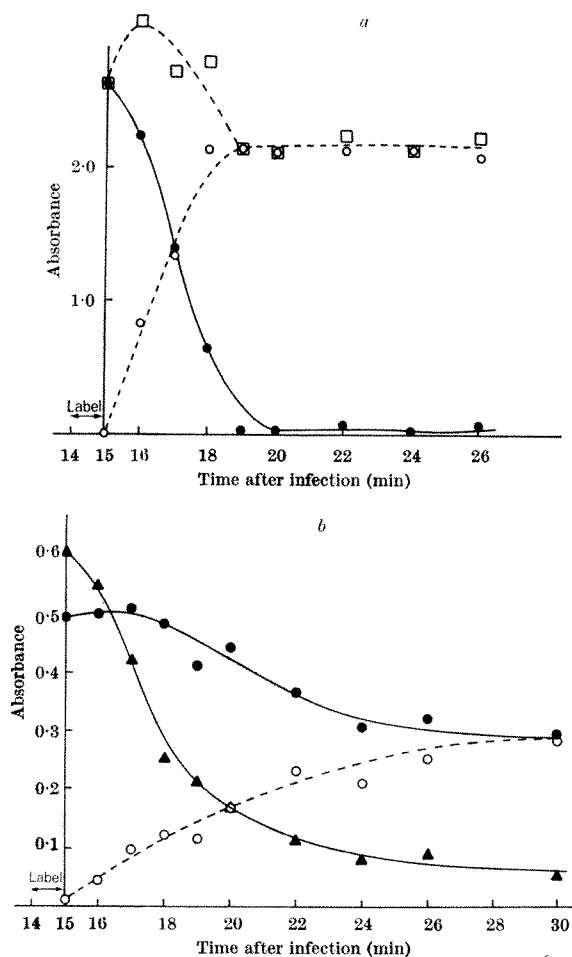


Fig. 5. Kinetics of cleavage of P23, P22 and IP. The kinetics of cleavage were measured using a microdensitometer (double beam recording microdensitometer, Joyce-Loebl) to record the autoradiogram. The exposure of the autoradiogram was chosen so that the absorbance of the band to be measured did not exceed 1 unit. The abscissa represents the integrated absorbance over the relevant peaks. *a*: ●—●, integrated absorbance over the P23* peak; □—□, integrated absorbance over the P22* peak; ○—○, total absorbance in P23 and P22*. *b*: ▲—▲, integrated absorbance over peak IP; ●—●, integrated absorbance over peak P23; ○—○, integrated absorbance over peak P22.

at late times (after 17 min). Phage assembly starts at about this time and it is therefore thought that the cleavage of IP is linked to phage assembly.

Cleavage of P24. P24* (mol. wt 43,500) appears coordinately with P23* and P22* (Fig. 4) (the kinetics are not plotted). The precursor product relationship $P24 \rightarrow P24^*$ is more difficult to demonstrate, because P24 migrates only slightly faster than P23*, but the following experiment proves that P24 is missing in a pulse-chase wild type lysate. P24 separates somewhat better from P23* in a gel of lower concentration. The samples of the pulse-chase experiment were analysed on 8 per cent acrylamide gels and four time points are presented in Fig. 6. P24 is easily distinguished from the small amount of P23* existing immediately after the chase of the radioactive label (Fig. 6, 15 min). P24 disappears at the same time as P24* appears while P23* increases. One might argue that P24 is obscured by the heavy P23* band. This possibility was excluded by adding a lysate (23-defective lysate) containing P24 to the final samples of the pulse-chase experiment. P24 was then detected and it can be concluded that measurements of P24 are reliable, thus showing that P24 most likely gives rise to P24*. The integrated absorbance values over these two bands are about equal, but the small loss of protein weight by the $P24 \rightarrow P24^*$ reaction is not likely to be detected by the densitometric measurements. P24 could not give rise to IP*, because the total label in IP* is two or three times larger than that of P24 and P24 is synthesized late. Moreover, the precursor relationship of $P24 \rightarrow P24^*$ is considerably strengthened by recent observations on head maturation genes (my unpublished results). P24 does not seem to be cleaved at all in 50-

defective cells and, indeed, no P24* is found. Cleavage of P23, P22 and IP does occur in 50-defective cells, although at a reduced rate.

Fate of the Small Fragments

Where are the small fragments of these cleavage reactions? The expected molecular weights for the small fragments stemming from P23, IP and P24 would be about 10,000, 2,500 and 1,500 respectively. Peptides of this size are not sieved on 10 per cent acrylamide gels and migrate with the marker dye (unpublished results of U. K. L. and J. V. Maizel). Attempts to find at least the 10,000 molecular weight fragment from P23 on gels of higher acrylamide concentration have failed. Possibly the fragments are further broken down to undetectable sizes. Fragments of P22 also have not been detected. Acid-soluble components which are derived from an acid-insoluble precursor are known to exist in T4 infected cells¹⁷. Two are associated with the phage particle and they are released with the DNA from the head upon osmotic shock¹⁷. The genetic determinant of one of these internal peptides has been mapped recently and lies in the neighbourhood of genes 20 and 21 (ref. 18). My results definitely rule out gene 20, which is incorporated unmodified into normal phage. Unfortunately, I have not discovered the product of gene 21 in the gel pattern. These results do not rule out the possibility that one of the internal proteins is derived from the small cleavage fragment of P22, P23 or P24. The appearance of the internal peptides seems indeed to be coordinated with the cleavage reactions of P23, P22 and P24. Genes 20, 21, 22, 23, 24 and 31, which affect the cleavage of P23, P22 and IP, are known also to affect the appearance of the internal peptides¹⁷.

It has been pointed out to me by S. Brenner and A. Stretton that the cleavage point must occur at the N-terminal end of the P23 protein. In establishing the co-linearity of gene 23 and its polypeptide⁴, they observed peptides in 23-defective lysates which contain the amber fragments, but these are absent in wild type lysates or in purified phage particles. These peptides may be derived from the N-terminal end of the amber fragment, which is cleaved off in the protein P23*. These observations also suggest that the small cleavage fragment, with an expected molecular weight of about 10,000, is fragmented to even smaller pieces.

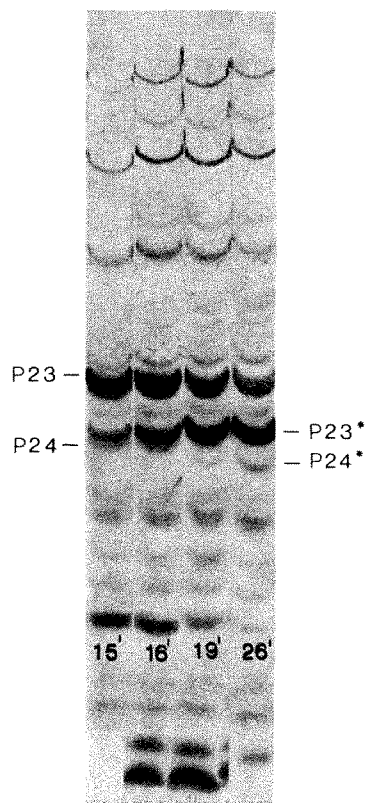


Fig. 6. Cleavage of the product of gene 24 (8 per cent acrylamide gels). Some samples (15, 16, 19 and 26 min) of the pulse chase experiment of Fig. 4 were analysed on 8 per cent acrylamide gels. Only the relevant part of the gel pattern is shown.

Cleavage occurs in a Large Structure

The observation that all the proteins P20, P21, P22, P23, P24 and P31 are required for efficient cleavage of P23, P22, P24 and IP suggests that these precursor proteins aggregate first to form an oligomeric structure, and are cleaved subsequently, rather than being cleaved first, and then assembled. The following experiment supports this view. The experiment is based on the observation that most of the precursor proteins are soluble and monomeric in SDS at room temperature, but that phage particles are not totally disrupted, as is also true in urea¹². The gel pattern of purified phage treated with SDS at room temperature is compared with completely degraded phage, boiled for 1 min in SDS (Fig. 7a and b). Only a small fraction of P23* is extracted from phage with SDS at room temperature. Most of the proteins stay at the top of the gel or enter the gel as high molecular weight aggregates. Some proteins are not extracted at all. Note, however, that a few proteins are almost quantitatively extracted from the phage particles. The samples from the pulse-chase experiment treated in SDS at room temperature only are shown in Fig. 7. P23 disappears with time but no P23* appears, suggesting that P23 enters an SDS-resistant structure before being cleaved. Of course, these experiments cannot rule out the possibility that P23* is converted to an SDS-resistant structure so rapidly that it is not detected. A high molecular weight protein appeared at the top of the gel, which could be an aggregate of P23 but only accounts for part of the label which disappears from P23. Most of the label stays at the top of the gel. The molecular weight of this structure must therefore be greater than 300,000, for such a molecular weight is excluded from these gels (unpublished results of U. K. L. and J. V. Maizel). It is possible that this structure has a capsid-like shape. IP* and P24* are not resolved in these gels. This is because of the high salt concentration in these samples ('M9' growth medium) which impairs the resolution of the gel in the low molecular weight region.

Maturation of the Head

My experiments demonstrate that the assembly of the head of bacteriophage T4 is not a simple, straightforward self-assembly, because several structural proteins are chemically altered at some stage of assembly. The uncleaved precursor protein P23 can, however, be polymerized into single and multilayered polyheads and τ -particles, if its cleavage is blocked as a result of mutation.

Investigations of genes 2, 4, 13, 14, 16, 17, 49, 50, 64 and 65, which supposedly control late steps in head formation, are in progress. It is interesting that the cleavage of P23, P22, P24 and IP seems to be normal in cells infected with phage carrying mutations in these genes with the exception of genes 2, 50 and 64 (my unpublished results).

Why are these structural head components cleaved? The finding that IP gives rise to an internal IP* sheds light on a possible consequence of the cleavage reactions. Internal proteins are thought to bind to DNA and the cleavage reactions may possibly trigger the necessary DNA-protein interactions, which result in orderly packing of the DNA within the shell of proteins. The following model may be proposed. The proteins P20, P21, P23, P24, P31 and IP form an intermediate structure (SDS-resistant) which combines with an end of a DNA strand. Cleavage of P23, P22, P24 and IP proceeds from the DNA attachment site. During this process more and more DNA binding sites may be formed at the inside of this structure, perhaps by the formation of IP*, thus winding up the DNA strand successively. The small acid-soluble peptides formed during this process may also interact with the packed DNA¹⁷ to neutralize charges. My results do not decide whether DNA packing proceeds simultaneously with the polymerization of the head membrane or follows thereafter.

I thank Dr A. Klug for encouragement and facilities, my colleagues J. King, J. Maizel and S. Altman for many valuable suggestions (J. Maizel in particular for advice on gel techniques and J. King for help in preparing the manuscript), and S. Brenner (among others) for critically reading the manuscript. U. K. L. holds an EMBO fellowship.

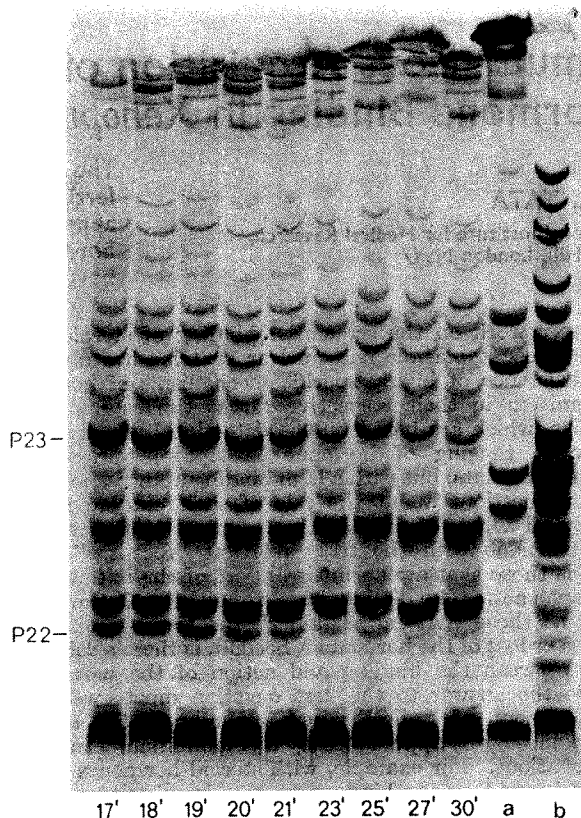


Fig. 7. Chase of P23 into a product stable to SDS at room temperature. A culture was infected and pulse labelled as described in Figs. 1 and 3 with the following changes. The culture was grown at 30° C, infected with wild phage, and labelled for 2 min from 15–17 min after infection. The samples were mixed at room temperature with an equal volume of twice concentrated "final sample buffer" without previous dialysis. The samples were then directly applied to the gels without being boiled. The sampling time is indicated at the bottom of the gels. As a control a purified phage preparation is analysed. a, Phage treated in SDS at room temperature only; b, phage boiled in SDS for 1.5 min.

Note added in proof. During the preparation of this manuscript I was informed that the alteration of P23 has also been observed by other workers: E. Kellenberger and C. Kellenberger-van der Kamp, *FEBS Lett.*, **8**, 3, 140 (1970); R. C. Dickson, S. L. Barnes and F. A. Eiserling, *J. Mol. Biol.* (in the press); and J. Hosoda and R. Cone, *Proc. US Nat. Acad. Sci.* (in the press).

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Simultaneous Acquisition of Metamorphic Response and Hormone Binding in *Xenopus* Larvae

by

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The acquisition of early metamorphic competence (36–60 h after fertilization) of developing *Xenopus* larvae is accompanied by the appearance of strong, temperature-sensitive binding sites for thyroid hormones. The findings have general relevance to the study of hormone "receptors".

RECENT work on the sequential binding of steroid hormones to cytoplasmic and nuclear proteins has revealed the existence of highly specific mechanisms of hormone recognition in their respective target tissues^{1,2}. These studies, which are based on the detection by radioactive hormone tagging of hormone "receptors" present in minute amounts, but with a high binding affinity, pose two major questions about the role of such binding in the biological action of the hormone. Can the interaction of the hormone with the truly relevant "receptor" sites, that is those that are an integral part of the initiation of hormone action, be distinguished from those that are not, such as those that may "buffer" the tissue against an excess or loss of the hormone? Second, is there a relationship between the binding and action of the hormone? While the answer to the first question must await the development of radically new techniques, the second question may be approached by a combination of indirect observations. For example, with steroid hormones it has been shown that the binding to cytoplasmic and nuclear components is abolished by compounds with strong anti-hormone properties^{3,4}. Another valuable approach in this direction, and one which has not yet been exploited, may be a developmental one. I describe here initial studies on hormone-binding during the early developmental stages of *Xenopus* larvae which reveal that whole embryos acquire an intense capacity to bind thyroid hormones at the same time as an early metamorphic competence as judged by biochemical responses of the organism to these hormones.

Earlier observations⁴ have shown that the developing larva of the clawed South African toad, *Xenopus laevis*, is unresponsive to the obligatorily metamorphic hormones, L-thyroxine (T_4) and 3,3',5-triiodo-L-thyronine (T_3), for about 40–60 h after fertilization. After this period the larva acquires competence in that it now responds to the hormone in a very abrupt fashion. Competence of response has been assessed in a number of biochemically ways such as changes in rates of synthesis of nucleic acids, proteins and phospholipids, PO_4^{3-} uptake, hydrolase induction, or—after a longer period—morphological changes characteristic of normal metamorphosis. I decided to follow the capacity and nature of thyroid hormone binding before and after the acquisition of metamorphic competence. There is virtually no type of cell in the pre-metamorphic tadpole that does not respond directly to thyroid hormones^{5,6}, and I therefore first attempted to study the larva as a whole. Furthermore, I have assumed that the diversity of biochemical and morphological responses of different cell types arises from the diversity of their commitments for further development by partially differentiated cells rather than from a diversity in the types of "receptors" or binding components.

The experimental design was based on the demonstration of acquisition of competence to respond to thyroid hormone by developing *Xenopus* larvae⁴. All conditions for ovulation, fertilization, growth and feeding of larvae were the same as described earlier⁴. Fig. 1 depicts a typical experiment in which binding of trace amounts of ^{125}I - T_3 was measured in *Xenopus* embryos at different stages of development. There was a marked increase in the affinity for the hormone of the developing organism between 40 and 60 h after fertilization (Nieuwkoop–Faber stages 34–41).

The increase in binding was most noticeable when expressed per embryo although quite substantial in terms of mg wet weight. The stages 36–41 of development are those stages which have been earlier characterized as the period of acquisition of sensitivity of the embryos towards thyroid hormones⁴. At all stages of early development the uptake of radioactive T_3 proceeds in a linear fashion for nearly 2 h at 20°.

In the next series of experiments the total capacity (as opposed to affinity) of the larvae to fix the hormone and its derivatives was determined by measuring the uptake of the radioactivity in the presence of increasing amounts of the non-radioactive compound. It was thus found that for both T_4 and T_3 the binding capacity increased rather abruptly and continued to do so for a period of time (Fig. 2). The binding capacity of the larvae increased from about 2 nmoles of T_4 /mg larval protein at 1 day after fertilization to over 25 nmoles and 45 nmoles at 3 and 6 days, respectively. The binding capacity for T_3 (Fig. 3) and T_4 (not shown in Fig. 3) exhibited a linear dependence on the amount of larval protein at all stages of larval development. During the early period of develop-

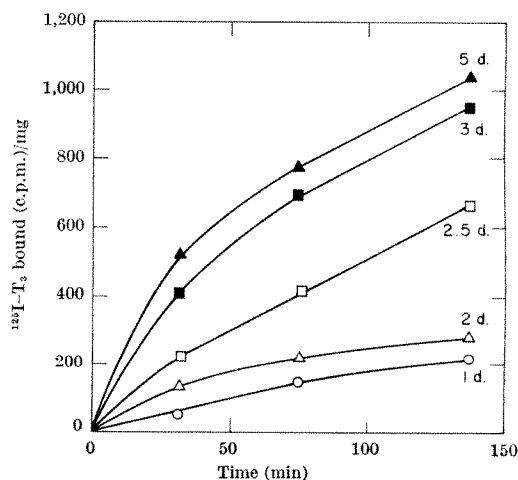


Fig. 1. Binding of a tracer (8×10^{-9} M) amount of ^{125}I - T_3 by *Xenopus* larvae at different stages of development (expressed as time after fertilization). Except for the one-day stage, a total of 200 mg of washed larvae was put in 50 ml. of water containing 8 μ Ci of ^{125}I - T_3 (0.4 nmoles) for different periods of time at 20° C. 500 mg of one-day embryos was used to allow for the large amount of jelly still present at this stage. At the end of different times of incubation the larvae were processed for bound ^{125}I - T_3 determinations. The larvae at different stages of fertilization were carefully washed in distilled water, and a variable number but a fixed weight of animals (200–500 mg) exposed to ^{125}I or ^{131}I -labelled T_4 , T_3 , or their analogues, for different times at controlled temperatures. The ^{125}I -labelled hormones were purchased from the Radiochemical Centre, Amersham; the ^{131}I -labelled compounds were prepared by radiolodination. Glassware was replaced with teflon or polypropylene apparatus for the pipetting and handling of the radioactive hormones because of the high affinity of glass for the hormones⁷. The binding of radioactive hormone was assessed by five quick washes of the larvae with water containing 10^{-4} M KI and homogenizing them in a teflon disintegrator with 1–5 ml. of 0.15 M NaCl. The radioactivity was determined in a Packard 'Autogamma' well-type scintillation spectrometer. In some experiments, ^{131}I was also measured in the homogenate, the 105,000g sediment and supernatants, as well as in the precipitate and supernatant obtained after treatment and washing of the larval homogenate with 5 per cent trichloroacetic acid. The larvae at each stage of development were obtained from different batches of eggs fertilized at different intervals of time so that the experiment was performed at the same time. Stages of development: ○, 1 day; △, 2 days; □, 2.5 days; ■, 3 days; ▲, 5 days after fertilization.

ment the larval protein and DNA content change in different ways. In the first 2-3 days after fertilization there is a very rapid increase in DNA content of the larva, followed by a more gradual change. On the other hand, the protein content dropped initially because of resorption of yolk and surrounding jelly but increased quite rapidly after 3-4 days, that is, following the feeding stage of the larvae. Thus, the pattern of increase in hormone binding capacity varies according to whether the results are expressed per unit protein or DNA (Fig. 4). It seems that initially the appearance of additional binding sites is a function of appearance of new cell population followed by an increase geared to the overall accumulation of proteins.

The binding is specific with respect to the biological activity of the analogue used as shown in Table 1. Thus the biologically more active analogues^{5,8}, T_3 and its propionic acid analogue were more avidly bound than the inactive analogues, 3,5-diiodo-L-thyronine and 3,5-diiodo-L-tyrosine. There was only a relatively small difference in binding affinities of the different compounds at 1.5 days after fertilization, but the difference between the biologically active and inactive analogues became more

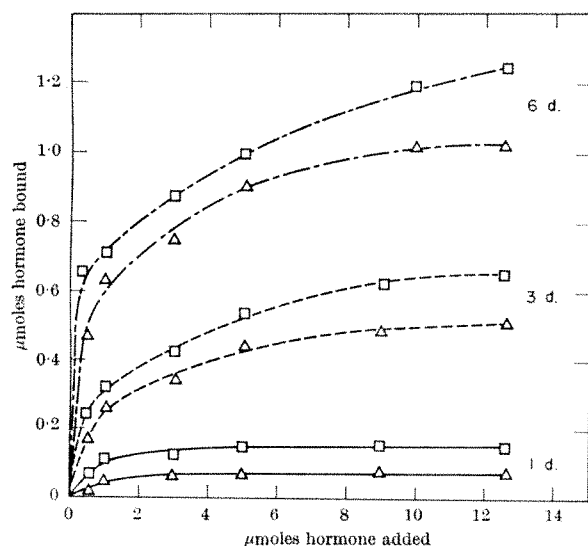


Fig. 2. Capacity to bind ^{125}I -labelled T_4 (□) and T_3 (Δ) by *Xenopus* larvae obtained at 1 (—), 3 (---) and 6 (— · —) days after fertilization. 500 mg of 1-day embryos and 225 mg of 3 day and 6 day stages were kept in 20 ml. of water containing $10\ \mu\text{Ci}$ of either hormone along with the different amounts of non-radioactive hormone, as indicated. The larvae were exposed to radioactive hormones for 40 min at 20°C . All other details as in Fig. 1.

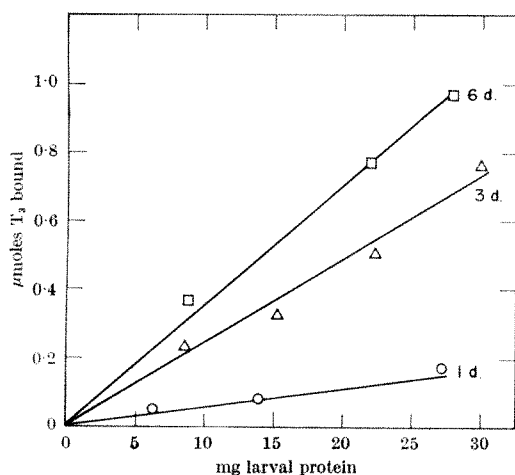


Fig. 3. Dependence of hormone binding capacity on the amount of larval protein. One (○), 3 (Δ) and 6 (□) day old larvae in different amounts were immersed in 30 ml. of water containing $2 \times 10^{-4}\ \text{M}$ ^{125}I - T_3 ($10\ \mu\text{Ci}$) for 45 min at 19°C .

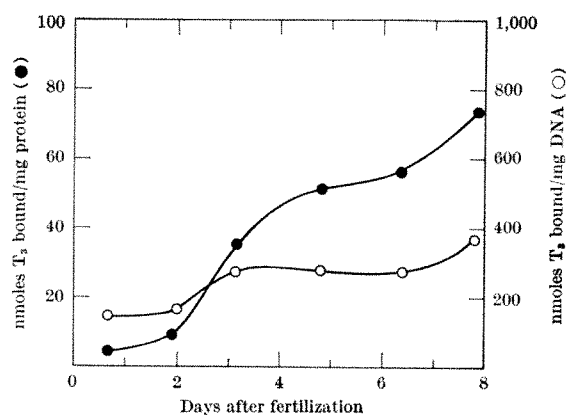


Fig. 4. Increase in T_3 binding capacity, expressed either per mg protein (●) or DNA (○) in larvae obtained at different times after fertilization. 450 mg of larvae at the one-day stage and 200-270 mg at other stages were kept for 40 min in water containing $2 \times 10^{-4}\ \text{M}$ T_3 ($8\ \mu\text{Ci}$) at 20°C .

apparent at 3.5 days. In saturation experiments, I found that the binding capacity was highest for T_4 , as is the case for mammalian blood and tissue proteins^{7,8}. Another type of experiment which also revealed chemical specificity of hormone-binding was competition by a large excess of unlabelled analogue added at the same time as the labelled hormone (see Table 2). The labelled hormone could only be displaced from the larval binding sites by analogues which are known to have high metamorphosis-inducing activity^{5,9}. Similar results in the binding of steroid hormones to their target tissues^{1,2} have been interpreted to reflect physiological relevance of the binding process.

In analysing the time-course of T_4 binding to larvae of different ages, I found that even very young larvae showed a substantial capacity to bind the hormone before they reached the stage when competence to respond to the hormone is reached⁴. It was thus necessary to decide whether this represented a non-specific absorption of hormone to sites present at all stages of development, or binding to physiologically relevant binding sites that may appear only at the time the organism shows a response to the hormone. While no direct and conclusive experiment could be designed to answer this question, I decided

Table 1. RELATIVE BINDING AFFINITIES OF *Xenopus* LARVAE FOR T_4 AND ITS BIOLOGICALLY ACTIVE AND INACTIVE ANALOGUES

Analogue	Potency ^{5,9}	Developmental stage (days)	Analogue bound (μmoles/mg)
L-Thyroxine (T_4)	High	1.5	240
		3.5	895
D-Thyroxine	Slight	1.5	332
		3.5	407
3,3',5-Triiodo-L-thyronine (T_3)	High	1.5	133
		3.5	769
3,3',5-Triiodothyropropionic acid	Very high	1.5	187
		3.5	1,463
3,5-Diiodo-L-thyronine	None	1.5	68
		3.5	135
3,5-Diiodo-L-tyrosine	None	1.5	98
		3.5	65

Larvae obtained at 1.5 and 3.5 days after fertilization were immersed in 20 ml. of water containing $5\ \mu\text{Ci}$ of labelled L-thyroxine or analogue ($5 \times 10^{-7}\ \text{M}$) for 10 min at 21°C . T_4 and T_3 were labelled with ^{125}I and the other analogues with ^{131}I .

Table 2. DISPLACEMENT OF LABELLED ^{125}I - T_4 BOUND TO *Xenopus* LARVAE BY BIOLOGICALLY ACTIVE AND INACTIVE ANALOGUES

Unlabelled analogue added	Concentration (M)	^{125}I - T_4 bound (c.p.m./mg)
None	—	2,355
L-Thyroxine (T_4)	8.0×10^{-7}	1,440
	5.2×10^{-6}	165
3,3',5-Triiodo-L-thyronine (T_3)	5.0×10^{-7}	804
	3.2×10^{-6}	249
D-Thyroxine	8.0×10^{-5}	1,085
3,3',5-Triiodothyropropionic acid	5.0×10^{-7}	1,160
	3.4×10^{-7}	311
3,5-Diiodo-L-thyronine	6.0×10^{-6}	1,980
3,5-Diiodo-L-tyrosine	2.5×10^{-4}	2,208

250-350 mg of 4.5 day old larvae were immersed in 30 ml. of water containing $20\ \mu\text{Ci}$ of tracer ^{125}I - T_4 ($6 \times 10^{-9}\ \text{M}$) and the non-radioactive analogue, at the concentrations indicated for 45 min at 18°C .

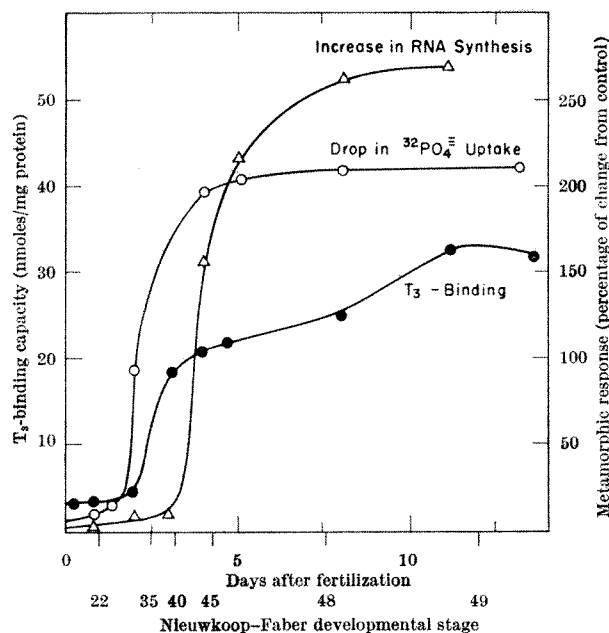


Fig. 5. Correlation between the appearance of temperature-sensitive binding capacity for T_3 (●) and the acquisition of a metamorphic response to thyroid hormone by developing *Xenopus* larvae. The temperature-sensitive binding component(s) was calculated by subtracting the binding capacity for T_3 at 25°C from that expressed at 5°C , as described in Table 3 and Fig. 4. The metamorphic response to T_3 is illustrated for the increase in the rate of RNA synthesis (Δ) or the diminution of uptake of PO_4^{3-} ions (\circ) when *Xenopus* larvae at different stages of development are exposed to $10^{-9}\text{ M } T_3$. The data on the early metamorphic response are taken from an earlier work⁴.

to exploit a difference in temperature sensitivity of binding at different stages of development which was initially observed by chance. Table 3 shows that whereas the hormone binding capacity at very early stages (before stage 30) was only slightly affected when measured at 5°C and 25°C , there was a marked increase in binding at the higher temperature in larvae beyond stages 36–40. It seems, therefore, that the acquisition of metamorphic competence is accompanied by the appearance of a temperature-sensitive T_3 -binding component. It is interesting that bullfrog tadpoles have been shown to exhibit temperature sensitivity during hormone-induced metamorphosis¹⁰, although it is difficult to establish a direct relationship between these two phenomena.

Assuming that the temperature-sensitive hormone-binding component(s) is related to metamorphic response, corrections were made for temperature in binding experiments shown in Figs. 1 and 2 and plotted against the known time-course of biochemical response of the developing larvae, derived from earlier work⁴. As shown in Fig. 5, there is a very good fit between temperature-sensitive hormone binding and the onset of metamorphic competence as judged by two biochemical indices (increase in the rate of RNA synthesis and decrease in PO_4^{3-} uptake). As the embryo developed, the correlation between bio-

Table 3. TEMPERATURE SENSITIVITY OF BINDING OF T_3 BY *XENOPUS* LARVAE BEFORE AND AFTER ACQUIRING METAMORPHIC COMPETENCE

Developmental stage (days)	Temperature ($^\circ\text{C}$)	$^{125}\text{I}-T_3$ bound (c.p.m./mg)
1.0	5	89
	15	125
	25	110
2.5	5	163
	15	295
	25	370
4.5	5	350
	15	879
	25	1,065
7.0	5	471
	15	1,208
	25	1,835

200–500 mg of larvae at different developmental stages were equilibrated at the different temperatures in 2.0 ml. of water for 30 min, following which $10\text{ }\mu\text{Ci}$ of $^{125}\text{I}-T_3$ ($2 \times 10^{-9}\text{ M}$) was added. The larvae were washed and homogenized after a further 30 min of incubation.

Table 4. DISTRIBUTION OF $^{125}\text{I}-T_3$ IN PROTEIN-BOUND AND PARTICULATE FRACTIONS OF *XENOPUS* HOMOGENATES AT 5°C AND 25°C

Fraction	Developmental stage (days)	Relative distribution		Fraction protein-bound (per cent)	
		5°C	25°C	5°C	25°C
Whole homogenate	1.5	100	100	47	56
105,000g pellet		23	31	53	64
105,000g supernatant		69	73	18	29
Whole homogenate	4.5	100	100	58	73
105,000g pellet		42	72	66	89
105,000g supernatant		54	24	32	59

Larvae obtained at 1.5 days and 4.5 days after fertilization were incubated with radioactive T_3 at 5°C and 25°C , in conditions described in Table 3. A part of the homogenate was separated into the particulate and supernatant fractions by centrifugation at 105,000g for 2 h and the protein-bound radioactivity determined by precipitation with 5 per cent trichloroacetic acid.

chemical or physiological responses and hormone-binding was less close. The latter function seemed to be acquired in two major bursts, one rapidly at 2–3 days after fertilization of the eggs and the other gradually at 6–10 days. The total protein content of the larvae increased very rapidly during the second period.

The reason for the two-step increase in affinity for the hormone may partly lie in different anatomical locations of the binding sites. While no systematic attempt was made to localize or fractionate binding sites, as has been done for steroid hormones, we have performed a preliminary autoradiographic analysis which may give a clue to this problem. Much of the hormone localized in the early forms of larvae was found to be in the region of the tail and gut, tissues that regress during metamorphosis. At later stages, and particularly in 6–10 days old larvae, a large amount of hormone was bound to other areas (such as the head) of the larva which would not regress during metamorphosis. In later stages of tadpoles, it is known that urea excretion (a hepatic function) is more sensitive to thyroid hormone than tail regression¹⁰.

A crude fractionation of the whole larval homogenate showed that much of the labelled hormone taken up from the medium was protein-bound (Table 4). A substantial fraction of the bound hormone was recovered in the 105,000g particulate fraction. The fraction of bound hormone recovered with the 105,000g sediment increased with development and it was this component that exhibited temperature-sensitivity and not that found in the supernatant. This finding suggests that at least a large proportion of the binding sites which appear at 2–4 days after fertilization may be localized in cellular structural elements and therefore do not merely reflect the appearance of circulating T_3 -binding protein. Furthermore, the blood of anuran tadpoles has not been shown to contain strong T_3 -binding globulins of the type found in mammals^{8,9}, nor are the circulating T_3 -binding proteins known to exhibit the temperature sensitivity that has been observed in these studies. In adult mammalian tissues also, a large part of endogenous or exogenous thyroid hormone is recovered in the 105,000g sediment, that is, the microsomal fraction^{8,11}. Of course, a more precise autoradiographic and subcellular localization, as has been attempted for steroid hormones¹², has still to be made for these thyroid hormone-binding sites. Even then earlier cell fractionation studies have indicated that a firm binding in itself does not necessarily indicate site of action⁸.

To conclude, our studies have shown that the acquisition of metamorphic competence by developing *Xenopus* larvae is accompanied by the appearance of thyroid hormone "receptors" which can be distinguished by temperature sensitivity from hormone binding components present at earlier developmental stages. These results have a wider physiological relevance, for a major requirement in understanding the action of hormones is the identification of cellular site(s) at which the hormone initially acts to trigger off a sequence of molecular events. It is generally recognized that the number of hormone-binding sites that are directly involved in the biological action of hormones must be very few relative to the total number of sites capable of interacting with the hormone. The outstanding problem is to find ways of distinguishing between these sites.

Although the work described here has to be extended a good deal further along the lines of the work on steroid hormone receptors¹, it nevertheless initiates a developmental approach to defining a physiological role for hormone binding by target cells.

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New Self-incompatibility Alleles produced through Inbreeding

by

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By drawing a parallel with microbial systems it is possible to envisage a scheme of intracistronic recombinations which could give rise to new self-incompatibility alleles.

ONE of the puzzles of higher plant genetics is the mechanism by which new self-incompatibility alleles are generated. These occur abundantly in natural populations, but extensive artificial mutation studies¹⁻⁶, involving several species and hundreds of millions of pollen grains, have produced no single clear-cut case of mutation of one active self-incompatibility allele to another. Mutations have been produced, singly or simultaneously, in the cistrons of the *S* gene complex which control pollen and stylar characteristics, resulting in the breakdown of self-compatibility, but none have been reported in the structural cistron which controls the specificity of *S* alleles. Several hypotheses have been proposed to explain this failure^{1,7-10}.

Occurrences of new specificities have occasionally been reported in experimental segregating progenies of self-incompatible plants in several species, but these could not unequivocally be proved to be new alleles and were assumed to be the results of contamination by stray pollen^{1,4,11-14}. Denward¹⁵, who worked on *Trifolium pratense* (red clover), was the first to suggest that new specificities regularly arose during inbreeding, and that the earlier reports of the occurrence of new alleles in inbred plants were not necessarily due to contamination. Because *T. pratense* is a widely cultivated pasture species in temperate countries, Denward's (from Sweden) could not reliably exclude the possibility of accidental contamination. But de Nettancourt and Ecochard¹⁶ have reported the occurrence of new *S* specificities in the selfed progeny of a clonal population of *Lycopersicum peruvianum*.

I wish to report the generation of unexpected specificities in an inbred plant of *Nicotiana bonariensis*. In a study of *S*-gene polymorphism in *N. bonariensis*¹⁷ a limited number of plants were grown in a greenhouse from seeds derived from four sources. Tests showed that altogether there were only six incompatibility groups, with between them only four different *S* alleles, suggesting that the seed from the four sources had a common origin. Thus the plants grown were probably fairly inbred. In one of the families of second generation plants which was grown from a compatible cross between two of the apparently normal plants, only four plants were obtained, because of the paucity of seed set and poor germination. One of these plants which was male fertile but female sterile was used as a male parent in several crosses with normal plants, originally with a view to studying the genetics of *S*-gene polymorphism and of female sterility. Several plants in the progeny were found to be of unex-

pected *S* genotypic constitution. Detailed descriptions of the parents and results of the progenies will be published later. I shall discuss now the phenomenon of the generation of new *S* alleles through inbreeding.

Involvement of Inbreeding

The unequivocal finding of the generation of unexpected *S* specificities in an inbred plant of *N. bonariensis*, taken in conjunction with the similar finding in *L. peruvianum*, supports Denward's idea that inbreeding is involved in the generation of new self-incompatibility alleles. But it does not necessarily support the view that inbreeding *per se* is responsible for the creation of new *S* alleles. There is considerable genetic and biochemical evidence that the specificity of *S* alleles is the function of the structural cistron of the *S* complex alone¹⁸⁻²⁷. Other major and minor genes, or their combinations, may drastically alter the strength of the expression of the *S* alleles, in certain cases practically obliterating their effectiveness, but there is no convincing evidence that, by themselves, they can produce new functional specificities. Furthermore, it is difficult to envisage how, in a cross-pollinated species, a new specificity produced by a rare polygenic combination would be present in different plants of a progeny and maintain itself from generation to generation.

Hypothesis

What is the role of inbreeding in the generation of new self-incompatibility alleles? One significant possibility is suggested by the work of Catcheside *et al.*²⁸⁻³², who found genes in *Neurospora crassa* which affect the frequency of recombination between allelic differences of a major gene. Four such specific recombination genes (*rec*) controlling intracistronic recombination have been discovered: *rec-1* controls recombination frequency between *his-1* (histidine-1) alleles, *rec-2* between *his-5* alleles, *rec-3* between *am-1* (amination-1) alleles, and *rec-4* between *his-3* alleles. The increase in recombination frequency may vary with different *rec* genes and may be very large, as in the case of *rec-1* where the factor of increase is commonly 15-20 or more.

Three properties of these *rec* genes seem to be significant in the context of the *S*-gene complex. (1) They are highly specific with respect to the major gene with which they are concerned. For example, *rec-1* which controls recombination in the *his-1* locus is insensitive to *his-3*, and similarly *rec-3* which controls recombination in the *am-1*

locus is insensitive to *his-1*. (2) Physically, they are all remote from the gene they control, that is they seem to be randomly located in the genome. (3) Of the allelic forms of these genes it is the dominant (*rec*⁺) alleles which always restrict the recombination frequency and it is the recessives (*rec*) which always ease the restriction and increase the recombination frequency.

These characteristics of the *icc* genes have led to the proposition that they are regulatory genes^{30,33}, analogous to those described in other organisms^{34,35}. Regulation by *rec*⁺ is most simply envisaged as acting directly at the gene level by the agency of a gene product which prevents either the formation of hybrid DNA or the correction of any consequently mispaired bases^{33,36}.

I propose that the principal mechanism of the generation of new self-incompatibility alleles lies in intracistronic recombinations in the structural cistron(s) of the *S*-gene complex, with a minor role of point mutations and deletions. The term "recombination" is used broadly and may include such a likely phenomenon as gene conversion which may or may not involve crossing over^{36,37}. I also propose that recombination in the structural cistron of the *S*-gene complex, like that in the *his-1*, *his-3*, *his-5* and *am-1* loci, is under the control of regulatory genes of the *rec* type found in *N. crassa*. In the case of the *S*-gene, however, I suggest that not one but several such regulatory genes are involved and have a cumulative effect.

In the outbred plants most of these genes will be heterozygous and, depending on the degree of heterozygosity, the expression of the dominant genes will suppress or nearly suppress recombination at the *S* structural cistron. Inbreeding produces homozygosity of these genes resulting in certain plants which have a considerable number of these genes in the homozygous recessive condition. In these plants, therefore, the suppression of recombination at the *S* locus is greatly reduced resulting in high recombination frequency and, consequently, new *S* specificities. Thus inbreeding is instrumental in releasing the structural cistron from the genetic suppression of recombination, whereas outbreeding maintains this suppression.

It is also possible that transcription as well as recombination of the structural cistron (perhaps of all structural cistrons of the operon as a whole) is under the control of these regulatory genes, as has been suggested by the study of *ad-3* locus in *Saccharomyces cerevisiae*^{33,38}. This may explain pseudo-compatibility which often results from inbreeding.

There are certain interesting evolutionary features of

this suggested mechanism of the genetic control of mutation at the *S* locus. It has an obvious selective advantage of increasing the rate of mutation when the range of *S* alleles is very small and results in inbreeding, and of decreasing or suppressing it altogether when the range is great and facilitates outbreeding. It thus has the potential to impart an extensive flexibility to the system. It ascribes a more defined function to at least a part of the polygenic component of the *S*-gene physiology, and indeed to a part of the polygenes in general. Such an evolution of the *S*-gene complex is not only consistent with all known facts regarding the *S* alleles, but is also in line with the operator-regulator model, considered to be one of the chief bases of the organization and control of major loci generally.

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Distinction between "Self" and "Not-Self" in Lower Invertebrates

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This study of an invertebrate tissue recognition system contradicts the notion that allogeneic ectoderm histo-incompatibility developed late in evolution. An "induced autodestruction" experimental system which has been developed could be useful in the elucidation of some obscure cytotoxicity phenomena.

UNTIL quite recently the acceptance by an invertebrate of material from another member of the same species was considered to be almost the rule^{1,2}. But exceptions to that rule are appearing in the literature; from the sponges upwards there are examples of a distinction between "self" and "not-self". Fusion between young sponges belonging to the same species fails³. Tissue incompatibility has been observed in various groups of cnidarians⁴⁻⁶. An allogeneic anamnestic response seems to be outlined in earthworms^{7,8}. Encapsulation of allo-

geneic material occurs in a gastropod mollusc⁹. No explanation of the mechanism(s) involved in these incompatibility phenomena has appeared. Any metazoan invertebrate is assumed to be able to eliminate or render harmless any foreign material entering the body¹⁰; it has also been suggested that, if there is any recognition system in invertebrates, it could only be non-immunological^{1,2,11}.

We have shown that *in vivo*⁶ and *in vitro*¹² tissue recognition takes place in the arborescent coelenterates

known as the Gorgonacea. Segments of branches taken from two individuals belonging to the same species fail to fuse, although experimental autografts succeed without difficulty. The conclusion that gorgonians distinguish self and not-self is confirmed by observations in nature where autografts occur very frequently. Natural allografts fail even in very favourable conditions⁶ (Fig. 1). We agree with Burnet² that "there is no *a priori* reason why two animals of the same species should not accept transplants from one another". Furthermore, neoplastic formations are not known to occur in coelenterates. But we think that in the frequent cases of gorgonians growing one against the other (Fig. 1) the safeguarding of proximo-distal morphogenetic gradients may be of biological significance in allograft failures.

An antibody-like substance has been found in sea anemones¹³, leading Phillips to conclude that the "immune mechanisms involved in these animals were not essentially different from those believed to be important in vertebrates". The appearance in gorgonian allografted hosts of a substance with passive haemagglutinating properties¹⁴ and suggesting an immune response had led us to similar conclusions, but these results have not been confirmed.

Gorgonian tissue is easy to culture¹²; hundreds of explants (0.4 mm²) can be prepared in a day. They are kept in small individual dishes containing 1 ml. of filtered seawater (FSW). Explants (1:1 in volume) to be tested for compatibility are superimposed one on the other. Xenogeneic explants (*Eunicella stricta* (A) and *Lophogorgia sarmentosa* (B)) are mutually damaged within 1-4 days of contact. The destruction is expressed by the collapse and the disintegration of the tissues. This histopathic effect also appears in allogeneic combinations, but usually after a longer delay. No histotoxicity is revealed when combinations of autochthonous tissues are prepared; these explants fuse and reorganize in spherules or in bean-shaped masses, and can survive indefinitely.

We have used a slight modification of this technique. The sizes of one of the explant's surfaces were in a ratio of 8:1, and thicknesses were similar. The larger explant was the killer explant and the smaller was the target. In these conditions (8:1), destruction was confined to the target tissue, the onset of toxic effects was early and the first macroscopic signs of disintegration were recorded in xenogeneic targets 10-12 h after exposure to the killer tissues. Total disintegration of the target occurred in 24-60 h according to the temperature and to the source specimens involved. An attempt to demonstrate some type of *in vitro* accelerated secondary response in gorgonians failed.



Fig. 1. Failure of fusion between homospecific gorgonians. A line separating the allogeneic tissues is clearly visible.

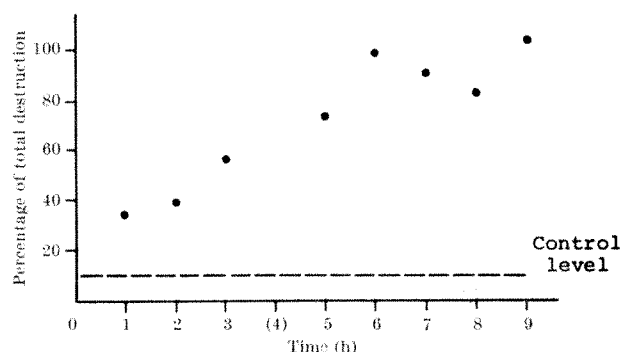


Fig. 2. Expression of the histopathic effect after contact (1-9 h) followed by the separation of killer and target.

An experiment was designed to specify more precisely the minimal time for the triggering of the disintegration, that is, the time during which the explants have to remain in contact to obtain detectable histopathy. The results are expressed according to the visual estimation (macroscopic) of the percentage of disintegrated tissue recorded at the nearest 10 per cent. The same expression has been used in Table 1. Three hundred and twenty xenogeneic explants (8:1, volume of species A and B) were superimposed at time zero, T_0 . At hourly intervals, $T_1, T_2, T_3, \dots, T_9$, forty of these explants were separated and kept apart in filtered seawater. The expression of histotoxicity was estimated 42 h after T_0 (Fig. 2). Results show that a minimal toxicity is manifest after about 1 h and gradually increases until 9 h. Results of a second experiment with samples taken at shorter intervals show that this minimal effect is already discernible after 30 min.

To discern the features of this tissue recognition system we studied the effects of antibiotics and of an amino-acid analogue on the expression of the *in vitro* xenogeneic and allogeneic histotoxicity. We used 60 µg/ml. of actinomycin D (Merck Sharp and Dohme), 100 µg/ml. of puromycin (Sigma) and 200 µg/ml. of *p*-fluorophenylalanine (Sigma) solutions in filtered seawater. Antagonistic explants in contact were maintained in actinomycin for longer than the time required for the expression of toxicity. Both explants remained intact.

The killer and the target were then treated separately: (a) killer explants were pretreated with actinomycin for 7 h, rapidly rinsed in filtered seawater and combined with untreated target explants; (b) target explants were treated in the same way and exposed to untreated killer explants. Controls included: (c) target explants in filtered seawater; (d) killer explants in filtered seawater; (e) target on killer explants in filtered seawater; (f) target on killer explant treated with actinomycin during the whole experiment. The same experimental design was used with puromycin and *p*-fluorophenylalanine. The results are shown in Table 1.

As Table 1b shows, when the target is pretreated the expression of histopathy is inhibited: 2 per cent, 0 per cent, 2 per cent. On the other hand, when the killer explant is pretreated (Table 1a) the histopathy index reaches 50, 63 and 47 per cent after 24 h, indicating severe damage. In another experiment using actinomycin, and by inverting the factors (species A being the target and B the killer), the indices were 20 per cent for the

Table 1. EFFECTS OF ANTIBIOTICS AND AN AMINO-ACID ANALOGUE ON THE EXPRESSION OF HISTOTOXICITY

	a		b		c		d		e		f	
Actinomycin D (for 7 h)	50	2	2	9	1	—	—	10	74	13	0	9
Puromycin (for 15 h)	63	14	0	10	10	—	—	12	84	25	5	10
<i>p</i> -Fluorophenylalanine (for 20 h)	47	12	2	10	7	—	—	9	28	7	4	8

Two sets of results are given for each of treatments a to f, which are explained in the text.

treated target, 76 per cent for the untreated one, 0 per cent for the treated killer and 1 per cent for the untreated one. Preliminary experiments with allogeneic explants of species *B* treated with actinomycin D showed similar results. Actinomycin D is known to inhibit synthesis of mRNA; puromycin and *p*-fluorophenylalanine interfere with protein synthesis. No information is available about their effect on the Cnidaria, but the wide zoological range of their activity supports the idea that their properties are the same in these invertebrates as elsewhere. The suppression of histopathology, simply by inhibiting action on the target, strongly suggests the existence of a mechanism involving an auto-destruction of the target explant triggered by the killer explant.

This interpretation is supported by results obtained by treating with actinomycin D a target previously in contact with a killer explant for 6 h. Such contact is sufficient (Fig. 2) to trigger more than 80 per cent destruction of the target. If a classical immunological system was involved, no synthesis would be expected to take place in the target, and destruction would be completed. But after treatment, no sign of histopathology appeared. The target was destroyed only if actinomycin was added after the twelfth hour following first contact. Thus the target seems to be the site of the active process. Species *A* and *B* have very small cells; photonic microscopy provided little information about the sequence of events in the destruction phenomenon.

Classical immunological systems are characterized by an induction phase initiated by antigenic material which penetrates an organism. This inductive phase is followed by a phase of synthesis of "responsive material" directed against the antigens and leading eventually to their inactivation. Destruction in gorgonians is apparently governed by a different mechanism, involving the "induced suicide" of the target explant while the killer seems to be passive. This system, although it can be regarded as unlike a conventional immune response, seems to be part of some kind of "pre-immunology". Two independent groups^{7,8,15-17} have interpreted the

accentuated rejection phenomena in earthworms as a type of anamnestic response. Worms are the most primitive group with a closed system of blood vessels, which is probably essential for an anamnestic mechanism to develop.

The "induced suicide" mechanism suggested here might explain the cell destruction observed in puzzling cytotoxic phenomena¹⁸, which in some cases could be mediated by "non-immune" mechanisms¹⁹. Cytotoxicity in these cases was suggested to necessitate cell membrane-bound action or it could be triggered by a diffusible substance²⁰. Whether there is activation in our system through a phase of the latter type is not known with certainty, although the destruction of remote gorgonian target tissues suggests diffusion.

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Correlation of Subpulse Structure in a Sequence of Pulses from Pulsar PSR 1919+21

by

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The fluctuation power spectrum of a sequence of momentary pulse energies formed using an integration gate narrower than the pulse width displays features not visible with a wider gate. The analysis indicates that the class two period is a function of phase within the pulse profile. The statistical constitution of the mean pulse is discussed.

PREVIOUS analyses of pulsar radiation have emphasized (a) average properties such as shape, strength and polarization of the pulses by averaging the data with the primary period (P_1) removed, and (b) pulse energy distributions and fluctuation spectra using a sequence of total pulse energies. The former analysis gives no information about how the average is related to the individual pulses and the latter gives no information about phenomena occurring within the pulse profile. This article forgoes these limitations and presents preliminary results of two statistical analyses of a two dimensional data array of pulse intensity versus pulse phase and pulse number for the pulsar PSR 1919+21*.

A Fourier analysis was performed on a sequence of momentary pulse energies, formed by integrating the received radiation intensity over narrow time gates which

were spaced by P_1 and hence corresponded to the same pulse phase for successive pulses. Repeating the analysis for independent phases converted the two dimensional data array to a two dimensional spectral array of fluctuation power versus frequency and pulse phase.

The second analysis converted the data array into an array of frequency of occurrence of a given intensity versus intensity and pulse phase. This is presented as a contour map of frequency of occurrence on an intensity versus pulse phase plane. These results are used to analyse the class two phenomenon which occurs in PSR 1919+21 and to discuss how the average pulse is constituted statistically.

Observations

The observations were made using the 1,000 foot spherical reflector at Arecibo Observatory with a linearly polarized, phase-correcting line feed operating at 319.5

* The standard notation for pulsars introduced by Turtle and Vaughan¹ is used. PSR 1919+21=CP 1919.

MHz. The received power was passed through a 100 kHz crystal filter, detected in a square law detector and smoothed with an RC time constant of 660 μ s. The detected signal was accurately proportional to the received radiation intensity. The output was sampled by an Adage A/D converter controlled by a CDC computer and written on magnetic tape. The sampling was done at 300 μ s intervals across a window of 120 ms centred on the pulse. The trigger pulse used to keep the window synchronized with the apparent pulsar frequency was created using a frequency synthesizer (HP 5100A) locked to the observatory time standard and a frequency counter (HP 245L) with scalar output. This time base was sufficiently accurate to keep the sampling synchronous with P_1 to within one sample in the 11 min block of data analysed.

Data Reduction

The block of 512 consecutive pulses used for this analysis was selected from several independent recordings of data at different radio frequencies on the basis of its having the largest signal-to-noise ratio. In preprocessing the data a baseline was subtracted from each pulse using the noise level in the window off the pulse. The block of 512 pulses was then ready for the transform and occurrence contour analyses.

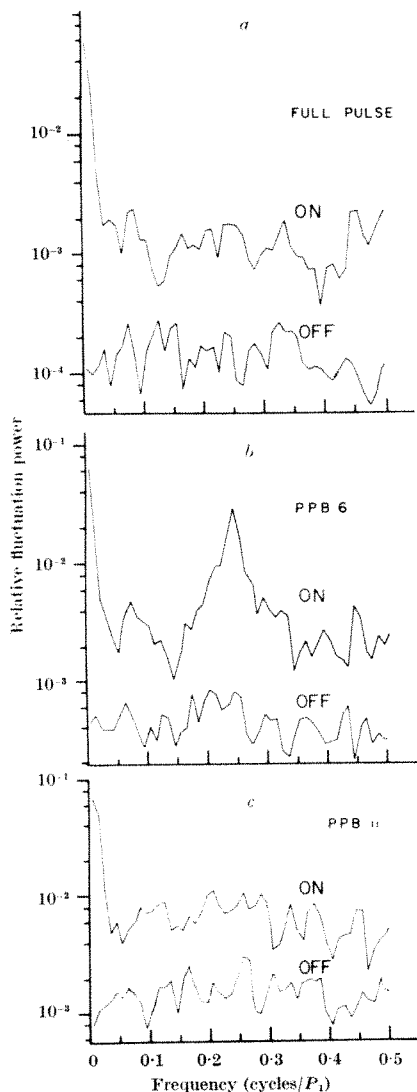


Fig. 1. The pulse energy fluctuation power spectra (ON) in cycles/ P_1 relative to the mean level squared, compared with spectra formed from similar data off the pulse (OFF). The ON spectra correspond to energy in (a) the full pulse (30 ms), (b) pulse phase box 6 and (c) pulse phase box 11.

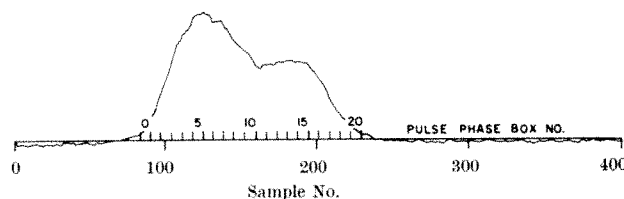


Fig. 2. Average pulse shape of PSR 1919+21 formed by the block of 512 pulses used in the analysis. Pulse phase box positions are indicated. Date, March 3, 1970. RF frequency, 319.5 MHz; bandwidth, 100 kHz; time constant, 660 μ s; sample interval, 300 μ s.

Two-dimensional Fluctuation Power Spectra

An "on" sequence of 512 numbers representing the overall pulse energies was formed by integrating the intensity over 100 contiguous samples in each window centred on the pulse for each of the pulses. An "off" sequence was formed in a similar manner using data in the window off the pulse. These were Fourier transformed using a Cooley-Tukey algorithm to produce a pulse energy fluctuation power spectrum similar to that presented by Lovelace and Craft². This transform, normalized to the value of the mean "on" level in the sequence squared and smoothed using a running mean over eight frequency components, is shown in Fig. 1a. It differs from that published by Lovelace and Craft by the absence of the feature they found at $\sim 1/(4.39P_1)$. The resolution in the spectrum is 0.016 cycles/ P_1 and the maximum frequency, the Nyquist frequency, is 0.5 cycles/ P_1 .

Twenty-one "on" gated energy sequences were then formed. Each pulse width was broken up into twenty-one pulse phase boxes (PPB) each containing seven samples (2.1 ms). The seven samples in each box were integrated to form a single number. The sequence formed by these numbers for a given PPB was the desired gated energy sequence. For each PPB an independent "off" sequence was formed in a similar manner. PPB 0 coincides with the leading edge of the pulse profile and PPB 20 with the trailing edge as shown in Fig. 2. The same Fourier analysis was performed on each of the twenty-one "on" and "off" sequences. Examples for PPBs 6 and 11 are shown in Figs. 1b and 1c. Each spectrum is normalized to its own mean signal level and is smoothed over eight frequency components. These spectra display four components as has been noted previously^{2,3}. There is (1) a low frequency fluctuation tail; (2) a white, or quasi-white, component; (3) a short period feature; and (4) a long period feature (visible only with less smoothing of the spectra). The measurement of the intense short period feature seen in the spectrum for PPB 6 was the preliminary objective of the analysis. This was a predictable result because the data were known to be very periodic due to the class two phenomenon (see the series of lines drawn to fit the evolution of correlated subpulse structure in a sequence of pulses in the paper by Drake and Craft⁴). The feature was not visible in the pulse energy fluctuation power spectra of Taylor *et al.*⁵ and Lang³. It was visible at a low level in the power spectrum for 6,144 pulses published by Lovelace and Craft² on a logarithmic scale. It is now clear that their response was due to a small residual modulation of the pulse energy which remained even after smoothing over several times P_2 (≈ 15 ms). A spaced overlay of the twenty-one smoothed power spectra in the vicinity of the short period feature is shown in Fig. 3. The scales are linear and vary from one spectrum to the next. The spectra, which were formed from independent sequences of numbers, evolve slowly as a function of PPB. This correlation of independent spectra is related to the typical width of the subpulses which participate in the class two phenomenon. Correlation over three to four PPBs each containing 2.1 ms of data implies a characteristic subpulse width of 7 ms in data with a 2 ms time constant. This interpretation is confirmed in the sequence of pulses published by Drake and Craft⁴ with a 1 ms time constant.

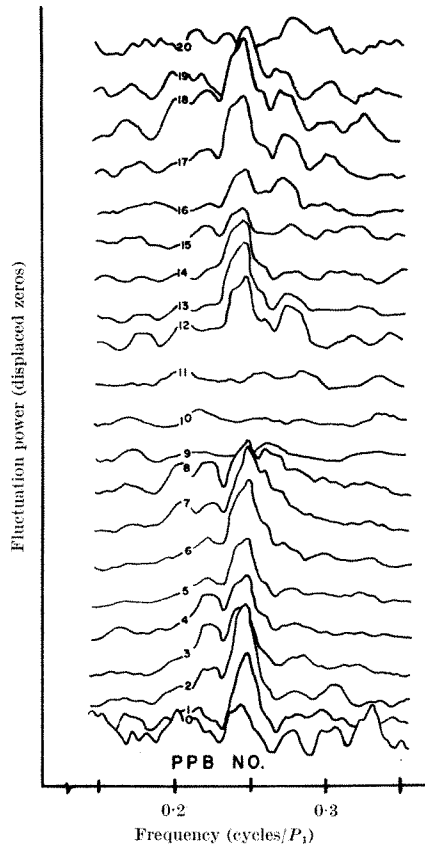


Fig. 3. Spaced overlay of segments of the spectra for 21 pulse phase boxes in the region of the short period feature described in the text. The power scales for the spectra are linear and vary from one spectrum to the next.

Another outstanding characteristic of these spectra is the disappearance of the short period feature in PPBs 9–11. This quantifies the effect also visible in the work of Drake and Craft⁴ that no subpulse peaks are found in the midpulse region (see their Fig. 2a). Finally, there is some evidence of higher harmonics of the short period feature. A secondary peak in the region 0.44 to 0.50 cycles/ P_1 (not shown) and those at 0.27 and 0.22 cycles/ P_1 (visible in Fig. 3) may result from harmonics of the feature at 0.24 cycles/ P_1 when aliasing is taken into account.

A summary of the variation of the centre frequency and strength of this feature as a function of PPB is given in Figs. 4a and 4b. To measure these quantities a baseline in the spectrum was determined on either side of the feature assuming the presence of a white component. The centre frequency was defined as the point at which equal energies occurred on either side in the feature measured above the baseline. The strength was defined as the amplitude ratio formed by dividing the square root of the integrated fluctuation power in the feature into the mean level of the signal for that PPB. Dividing this ratio, which in places exceeds unity, by the width of the feature in frequency components (typically 10–20), gives the fractional modulation of the mean level by the feature. The typical result is roughly 10 per cent.

An important reason for this analysis was to see if the phase variation of the predicted short feature could be used to quantify the class two phenomenon as an average property. In terms of the series of lines that are drawn to match the evolution of subpulses through a short sequence of pulse profiles, the centre frequency of this feature measures the average “vertical” spacing (P_3) of the lines and the gradient of the phase of the power components in the feature across the PPBs measures the “horizontal” spacing (P_2). The Cooley–Tukey algorithm receives a complex input array and produces a complex

spectrum with spectral components S_k derived from the summation in equation (1).

$$S_k = \sum_{j=1}^{512} N_j W^{-jk} \quad (1)$$

where $\text{Re}(N_j)$ = j th data point

$$\text{Im}(N_j) = 0$$

and $W = \exp(2\pi i/512)$, the principal 512th root of 1.

The phase for a given S_k is the angle ϕ_k of the phasor representing S_k on a complex plane.

$$\phi_k = \arctan [\text{Re}(S_k)/\text{Im}(S_k)] \quad (2)$$

If the data contain a periodic component with a phase angle at the origin of ψ , $\cos(2\pi f(k \cdot P_1) + \psi)$, then that frequency component in the spectrum will have a phase $\phi_{k*} = \psi$.

$$\text{Re}(S_k) = \sum_{k=1}^{512} \cos(2\pi f(k \cdot P_1) + \psi) \cos(2\pi \left(\frac{k}{512 \cdot P_1}\right) (j \cdot P_1)) \quad (3)$$

$$\text{Im}(S_k) = \sum_{k=1}^{512} \cos(2\pi f(k \cdot P_1) + \psi) \sin(2\pi \left(\frac{k}{512 \cdot P_1}\right) (j \cdot P_1))$$

For $k^*/512 \cdot P_1 = f$, the summations in equation (3) can be reduced.

$$\text{Re}(S_{k*}) = \frac{1}{2} \cos \psi$$

$$\text{Im}(S_{k*}) = \frac{1}{2} \sin \psi$$

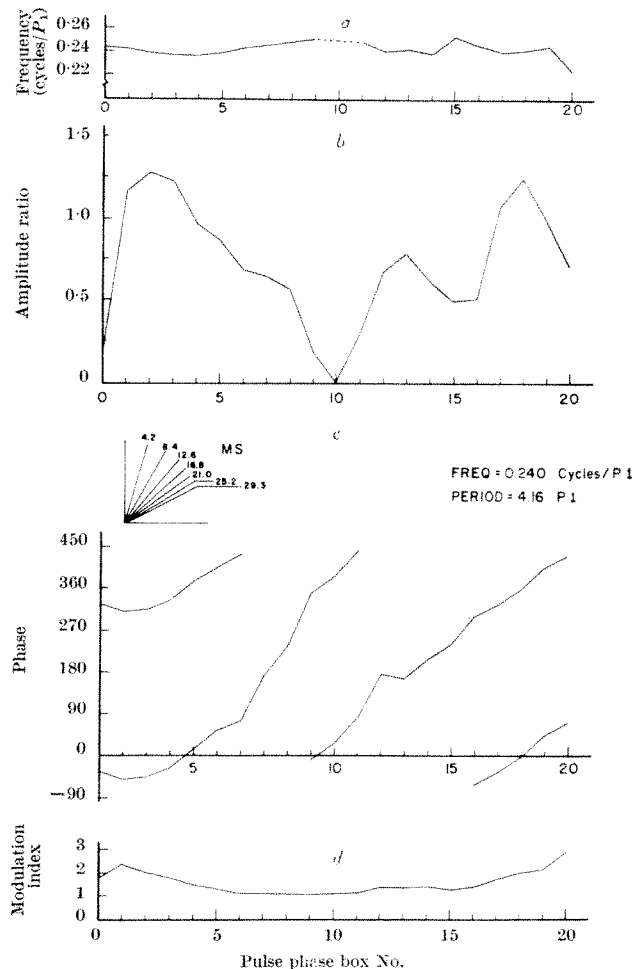


Fig. 4. Summary of the variations observed in the short period feature over the 21 pulse phase boxes. a, The centre frequency in cycles/ P_1 . The broken line corresponds to the region where the feature is undetectable. b, The amplitude ratio of the integrated strength of the features in each PPB spectrum expressed as a ratio of the mean level for that PPB. c, The phase of the frequency component $f=0.240$ cycles/ P_1 as defined in the text. d, The modulation index expressing the ratio of the fluctuation power to the mean level for each pulse phase box.

and therefore

$$\varphi_{k*} = \psi$$

As the correlated subpulses drift from the trailing edge of the pulse width to the leading edge over several pulses, the phase should increase with PPB. Such behaviour does indeed occur as shown in Fig. 4c, the phase against PPB for $f=0.240$ cycles/ P_1 , or $k*=123$. Adjacent frequency components have phase functions of identical shape with a constant offset. A phase increase of 360° indicates that one whole period of P_2 has been crossed in the PPBs and that the phase of the frequency components in the feature for succeeding PPBs is now referred to a new line of subpulses. The slope of the phase function is then a measure of the average subpulse spacing in the class two system of subpulses (P_2). A series of slopes are shown in the corner of Fig. 4c. The spacing is seen to vary slowly across the profile. It is 17.6 ms on the trailing edge where the subpulses first "appear", decreases to 10.7 ms in the midpulse region and then increases to 23.1 ms on the leading edge. This phase function and others suggest strongly that the subpulse spacing increases indefinitely as the subpulses "disappear" from the leading edge. Thus the "march" of the subpulses becomes infinitely fast as the subpulse leaves the leading edge. This inconstancy of P_2 is noticeable in the original data presented by Drake and Craft⁴ (see their Fig. 2a) and may be the cause for inconsistent measurements of P_2 (private communications from Drake and from Vitkevich), because the series of lines to fit the subpulse evolution are usually drawn as straight lines to facilitate analysis of the phenomenon. This result suggests that the "lines" of subpulses are actually S-shaped curves with a constant spacing due to the constancy of the centre frequency with PPB, but with a variable slope related to the variation in the gradient of the phase function over the pulse profile.

Modulation indices (m) for each PPB were computed from the twenty-one spectra and are plotted against PPB in Fig. 4d. Each is computed by finding the ratio of square root of the integrated fluctuation power in the entire spectrum to the mean "on" level. This curve will be discussed later.

Two-dimensional Frequency of Occurrence Map

A smoothed contour map of the frequency of occurrence of a given intensity (ordinate) for a given sample in the window (abscissa) in the block of 512 pulses is shown in Fig. 5. The intensity intervals correspond to two logical levels in the A/D converter and samples are spaced by 300 μ s. Typically 5 to 10 per cent of the samples on the pulse fall below the lower 10 contour while 10 to 20 per cent rise above the upper 10 contour. Cuts through the contours at various sample numbers were compared. The peaks of the resultant histograms follow the mean level closely. The other feature of these histograms is the high intensity side of the distribution. The small block of data prevented a highly significant analysis, but the histograms suggest an exponential tail with a slope dependent on the mean level, the slope being flattest where the pulse is strongest.

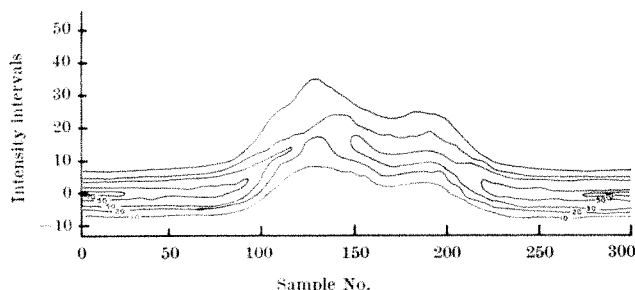


Fig. 5. A smoothed map of frequency of occurrence of a given radiation intensity for a given sample number.

Discussion

It is now possible to discuss how the average pulse is related to the individual pulses during a strong burst. The occurrence contour map indicates that the individual pulses are dominated by a constant component. The two-dimensional Fourier analysis indicates the presence of a second component, which is the system of subpulses participating in the class two phenomenon.

From Fig. 4b it seems that the subpulses are modulated by an independent envelope synchronized with P_1 . This envelope has two peaks separated by a null. One peak occurs on the leading edge of the average profile before the first peak; the other is centred in the trailing half of the pulse. The latter peak appears slightly double. This doubling can be explained perhaps by a broadening of the feature in the spectrum into the spectral region used to determine the baseline. Because the null corresponds to a region where P_2 decreases to 10.7 ms (see Fig. 4c), it is possible that the modulation disappears because of a merging of successive subpulses in that region of the pulse. On the basis of these two interpretations of the analysis it is possible to say that the subpulses are modulated by an envelope similar but not identical to that of the long term average pulse shape.

The modulation index variation shows an increase by a factor of two on the leading and trailing edges of the pulses where the mean pulse level is low. The subpulse contributions to that level must be large and infrequent. They are probably the result of an occasional subpulse marching beyond the normal confines of the pulse profile.

The contour map (Fig. 5), because it resembles the mean pulse shape, shows that the variations in the mean pulse across its width are caused by variations of the same shape in individual pulses rather than a variation in frequency of occurrence across the width of more uniform intensities. This suggests that a single phenomenon external to the radiation producing structure modulates the entire pulse. The interpretation that the subpulses are related to the radiation producing structure, and accelerate as they approach the leading edge of the pulse is consistent with this interpretation. The possible variation across the pulse profile of the exponential tail out to high intensities would indicate one property of the external modulating phenomenon.

Finally, there is a long period modulation (P_4) of the pulse energy. It appears in the spectra done for this analysis as two peaks at frequencies near 0.0088 and 0.017 cycles/ P_1 . This feature was first reported by Lang³ and has been discussed by Staelin *et al.*⁶ and Lang (unpublished). It is equally strong in both the non-gated and gated spectra. In particular, it is present in the PPB 10 spectrum where the short period feature vanishes, although that may be due to other reasons. Thus from this analysis, it is not clear whether the long period modulation is related to the correlated subpulse phenomenon⁶ or not (Lang, unpublished). If, in fact, P_2 is not a unique quantity, then there are obvious difficulties in trying to tie the periods P_1 , P_2 , P_3 and P_4 together as Staelin *et al.* have attempted to do.

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LETTERS TO NATURE

PHYSICAL SCIENCES

MIRVs and the Strategic Balance

MATHEMATICAL models are a useful aid in describing the strategic balance of deterrence, but must be used with caution. Bellamy¹ uses a model to show that, in certain circumstances, the fitting of multiple independently targetable re-entry vehicles (MIRVs) instead of single warheads on intercontinental ballistic missiles may make the strategic balance more stable. He calculates the number of warheads X_b belonging to side B which would survive a first strike by side A and could then be used against A's cities in a retaliatory second strike. N_b is the number of launchers possessed by B, each with reliability R_b , and with n_b warheads. A fraction f of A's N_a missiles are used for a first strike, and each of the n_a warheads has a probability C_a of destroying one of B's launchers. By assuming a situation in which A and B have identical parameters he obtains a simple expression

$$X_b = X_a = RnN(1 - C)^{fnr}$$

which can be maximized with respect to n . For plausible values of the parameters, the value of n corresponding to maximum X is greater than 1.

A practical question might be asked concerning the assumption that n MIRVs on one rocket are as effective as n single warhead missiles for the purpose of retaliation against cities. An alternative simple assumption is that the n MIRVs on one rocket are only as effective as one single warhead missile against cities, which has some justification on the grounds that the total megatonnage of explosive in the n MIRVs will almost certainly be less than that in the single warhead, and that it may not be possible to aim the MIRVs from one launcher at several widely dispersed cities.

The assumption that $N_a = N_b$ and that the other parameters are identical for A and B is too restrictive for a thorough examination of the stability of the strategic balance. G. D. K.^{2,3} has developed a model which allows A and B to choose different parameters, and a similar model has been used by Saaty⁴ to discuss the effects of multiple warheads.

Our model uses a graphical display to illustrate the state of the balance and hence does not have to assume symmetry to obtain a result. It introduces a "minimum deterrent" U_a , representing the minimum number of missiles which A would have to deliver to B's cities in order to deter B, and a corresponding U_b . Also, to allow for untargetable weapons like missile-firing submarines, a parameter k_b represents the proportion of B's missiles which can be engaged by A's missiles. Taking Bellamy's f and R to be 1, and using his notation, the equations showing the number of A's missiles (N_a) needed to reduce B's second strike strength below the deterrence threshold and still retain U_a to threaten B's cities are

$$\begin{aligned} (N_a - U_a)n_a &= SN_b k_b \\ N_b k_b (1 - C_a)^* + N_b (1 - k_b) &= U_b \end{aligned}$$

and there are similar equations describing B's ability to make a disarming first strike against A.

These curves divide up the domain of positive N_a , N_b , and produce a balance diagram which takes one of the forms shown in Fig. 1. In these diagrams, I indicates the area of mutual deterrence, II that in which A dominates and III that in which B dominates. IV, if it exists, represents a "pre-emptive area" in which the first attacker wins and V a subcritical area in which neither side has enough missiles to destroy the other. In area I, the stability of deterrence for any particular combination of A's and B's stocks, represented by a point P , is indicated by the distance of P from the boundary lines, for this shows how far stock levels can be changed without upsetting the balance.

The application of this model to the case considered by Bellamy is shown in Fig. 2. The parameter values used are $U_a = U_b = 50$, $C_a = C_b = 0.5$, $k_a = k_b = 1$. Curves defining the area of mutual deterrence for values of $n = n_a = n_b$ from 1 to 4 are shown. To avoid confusion the pre-emptive areas which exist when $n > 2$ are omitted. It can be seen that, as n increases, the area of mutual deterrence, and stability, decreases. If the original stocks were 700 each, as indicated by the point P , the situation is very stable when $n=1$ and remains reasonably stable up to $n=3$. When $n=4$, stability has almost disappeared, and when $n=5$, the situation is pre-emptive.

The situation when ABM defence is introduced is illustrated in Fig. 3. This starts with the same parameters as before but fixes $n=3$; the curve labelled "no defence" is therefore the same as that labelled $n=3$ in Fig. 2. To counter the use of MIRVs it is assumed that an ABM defence capable of destroying one out of two incoming warheads is deployed. If this defence is applied to protect cities only, the area of mutual deterrence is

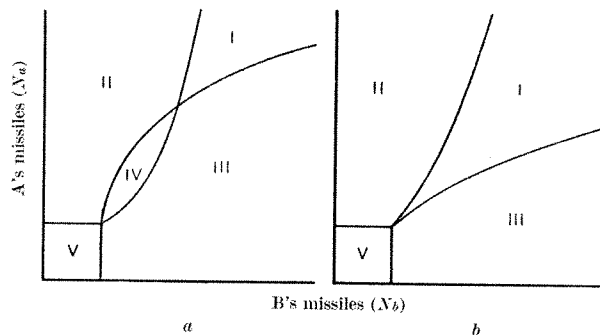


Fig. 1. Types of strategic balance diagram.

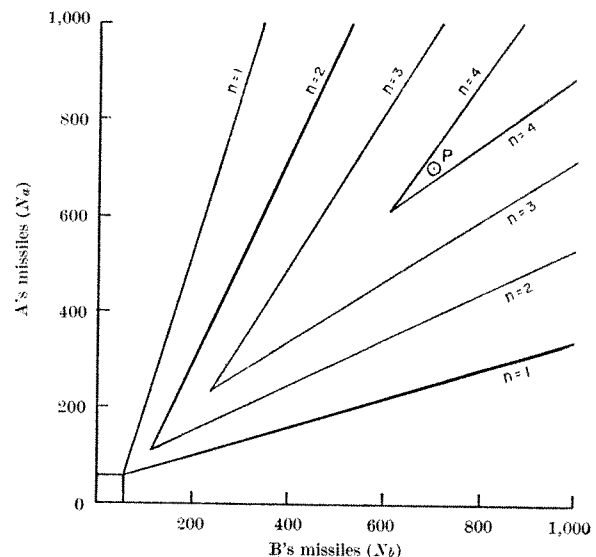


Fig. 2. Loss of stability as n increases.

greatly reduced; the point P , representing equal stocks of 700 missiles each, is now near the boundary. On the other hand, when the defence is used to protect missile launchers only, stability is greatly increased, almost to the level where no MIRVs and no defence are used; the case shown by the dashed lines. The asymmetric case where one side has the MIRVs but no ABM defence and the other ABM defence of launchers but no MIRVs is represented by taking one of the "defend launchers" lines with the opposite dashed line, indicating that such a situation departs only slightly from a symmetrical balance.

If we accept Bellany's assumption that the deterrent effect is proportional to the number of warheads rather than the number fired in retaliation, the balance diagram of Fig. 4 is obtained for the case with ABM defence. This demonstrates Bellany's point that MIRV can increase stability, because for values in the vicinity of $N_a, N_b = 40$ (point Q) there can be stability for $n=5$ but not for $n=1$ or $n=10$. For most of the domain of N_a, N_b , however, increasing n decreases stability.

These diagrams also illustrate the difficulties to be faced in an arms reduction programme, if a stable state

with large N_a, N_b , is to be kept stable while reducing the number of weapons.

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Modification of Stratospheric Properties by Trace Constituent Changes

It has been suggested that large quantities of water vapour and other pollutants introduced into the stratosphere by supersonic transporters may change the basic properties of the stratosphere¹. But what happens when large changes in trace constituent concentrations occur naturally, for example, after a volcanic eruption? The stratospheric temperature seems to increase by about 5° C and it remains higher than normal for several years. Some of the evidence for this is given in Figs. 1 and 2, which are based on daily upper air data from Australia and New Zealand. Monthly temperature means were formed for each month, then mean monthly values computed from data for the period 1958–62 inclusive. Deviations from these long term values were noted, and a three-month running average obtained. Fig. 1 gives the time variations of these deviations at selected stations. A major eruption of Mt Agung, Bali (8° S, 115° E), occurred on March 17, 1963, and there have been reports of other eruptions that year. Temperature at the low latitude stations increased almost immediately and remained higher than normal for over two years. Fig. 2 contains meridional cross-sections of the temperature deviations

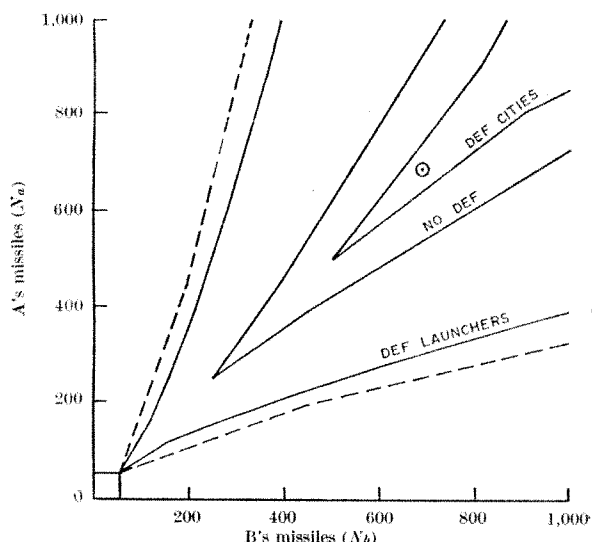


Fig. 3. Effect of MIRVs and ABM defence (deterrence dependent on number of missiles).

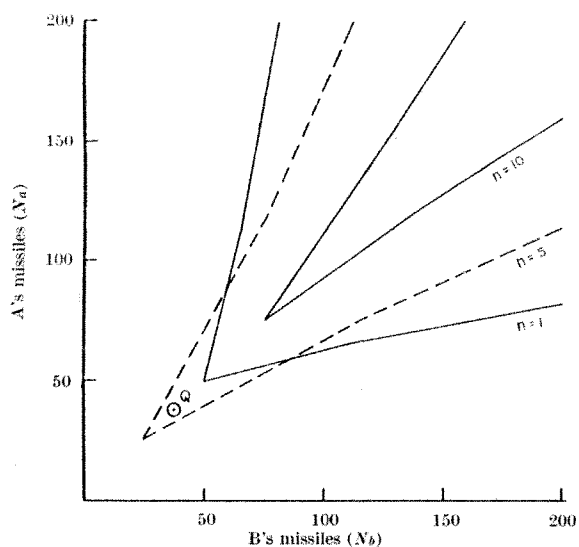


Fig. 4. Deterrence dependent on number of warheads (ABM defence present).

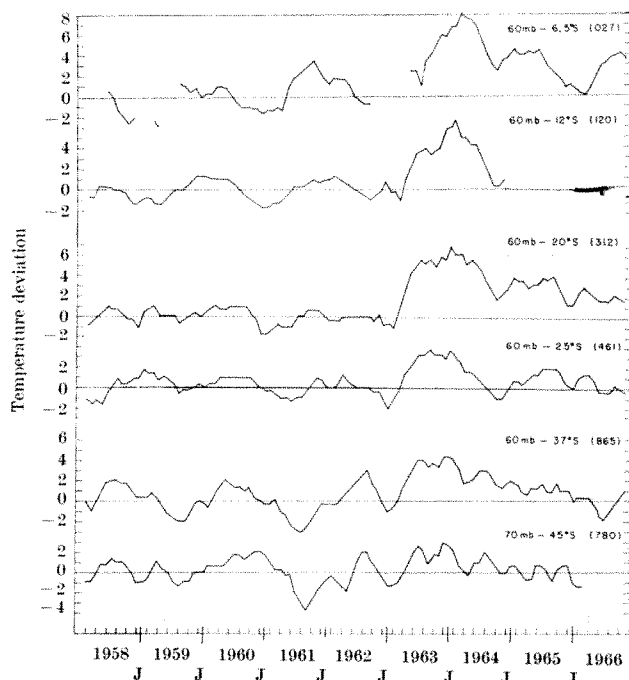


Fig. 1. Temperature deviations from monthly averages based on the period 1958–1962. Units: degree centigrade. Pressure level shown in millibars (mb). Australian stations 027: Lae (6.5° S, 147° E), 120: Darwin (12° S, 131° E), 312: Port Hedland (20° S, 119° E), 461: Giles (25° S, 128° E), 865: Laverton (38° S, 145° E). New Zealand Station 780: Christchurch (43° S, 173° E).

for particular months based on station data for the longitude region 110° E to 180° E. At the beginning of 1963, temperature was below the five year normal and the time variations had been following the characteristic southern hemisphere biennial pattern². The eruption interrupted this pattern. The cross-sections bear some resemblance to those for the products of nuclear weapons tests (for example, see the isolines of tungsten 185 radioactivity in ref. 3), as might be expected. The deviation pattern exhibits a maximum at low latitudes in the lower stratosphere and slopes downwards towards the pole, in some cases at a slope greater than that of the potential temperature surfaces. It is difficult to detect the changes, if any, below 300 mbar. While negative values predominate in the examples shown, it was not possible to draw in a -2° C contour. Similarly, at higher latitudes in the stratosphere the effect is uncertain; large deviations from average are more common there. It has been reported² that the Australian Weather Bureau changed its thermistor mounting at the end of 1962. Maps of temperature for January 1963 and 1964 show that the temperature change is present at other longitudes.

not discussed here. The height of those constant pressure surfaces above the dust layer was raised by up to 200 m in January 1964 at 50 mbar. With no obvious compensatory cooling at higher levels, although doubtless the circulation patterns there will adjust somewhat, one might expect to see larger densities measured by the satellite drag technique. I leave a search for this effect to others more conversant with the problems of satellite data.

Measurements of solar radiation at the surface¹² and scattered light from twilight¹³ have been used to trace the cloud from Bali. Temperature deviations may also be used where the data are adequate and we are working on this problem now, using also stratospheric mean wind charts for the period. To assist in this work we would greatly appreciate copies of any reports or reprints dealing with twilight glows, temperature anomalies or surface radiation anomalies. Many people noted the Krakatoa events (see Symons⁶) and the interest in theories of climatic change involving volcanoes was thereby heightened^{14,15}. Bali produced temperature changes in the stratosphere much larger than those normally considered in climatic change discussions.

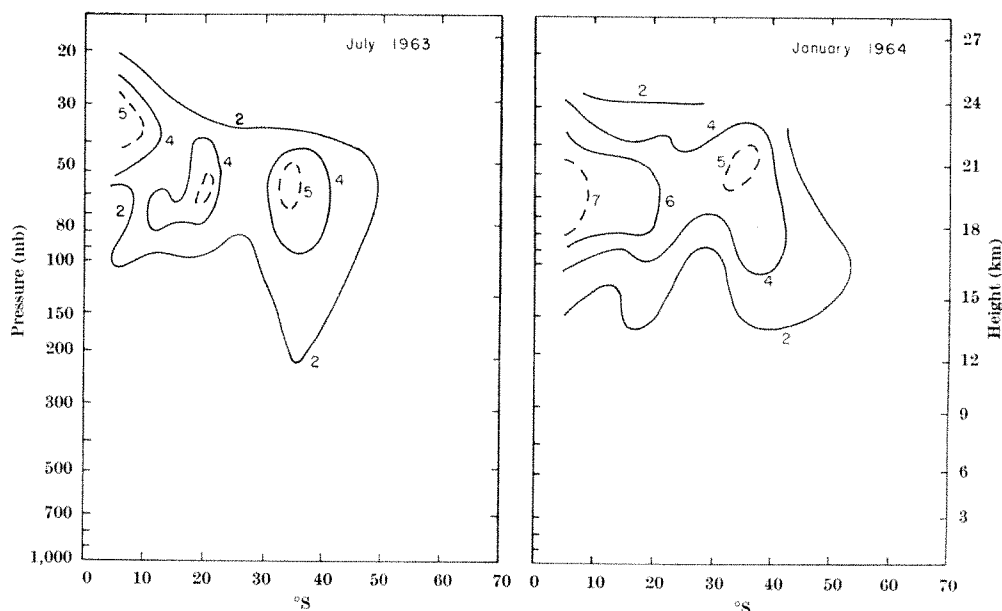


Fig. 2. Meridional cross-section drawn from data for stations used in Fig. 1 and additional stations. Isolines are temperature deviations, during month shown from monthly average based on 1958-1962. Units: degrees centigrade.

If temperature changes of up to 5° - 6° C above average are produced within about 2 months, as seen from Fig. 1, the equivalent heating rate is at least 0.1° C/day, which is a relatively large contribution in the lower stratosphere. The heating is probably caused by absorption of solar radiation by aerosols such as that demonstrated by Robinson⁴ for the troposphere. The exact nature of the particles is unknown, although Mossop⁵, who sampled them directly from an aircraft, comments that the dust seems to be coated with sulphuric acid. Volcanic gases contain large quantities of water vapour, carbon dioxide and sulphur dioxide, and fairly rapid formation of sulphuric acid has been suggested for the Krakatoa eruption⁶. A strong smell of sulphur was reported at Bali⁷. Several workers have suggested that the particles grow *in situ* from the gases introduced into the stratosphere^{5,6,8-11}.

Station data from the northern hemisphere tropics also show evidence of a similar temperature increase. An overall increase of 5° C in the layer 17-25 km over more than one quarter of the globe will evidently produce other geophysical changes. There are obvious items, such as changes in the mean wind field, the evidence for which is

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Evolution of Rifting in Africa

THE recent report by Girdler *et al.*¹ on the rift system of East Africa is of great interest because it presents a crustal model, based on new gravity observations, which suggests an intrusion of low velocity mantle material rising to the base of the sialic crust which underlies the eastern and western rift valleys of East Africa between 3° N and 9° S over an east-west width of about 1,000 km. The crustal model proposed is thus in a general way comparable with the postulated to underlie the worldwide mid-ocean ridge² and the Upper Rhinegraben³. Studies of seismicity, age of faulting, age of volcanoes and short and long wavelength gravity anomalies are interpreted by the authors as indicating attenuation of the African crustal plate and the early stages of its breakup. The structural behaviour of East Africa is compared with the conveyor belt mechanism of sea floor spreading from mid-ocean ridges as recently reviewed by Vine⁴ and rates of movement away from the rift axis of 0.5–2.0 cm yr⁻¹ are said to be indicated.

Such high rates of spreading are hard to reconcile with the geological picture of East Africa which is now known in some detail through the many map sheets published by the Geological Surveys of Kenya, Uganda and Tanzania. The terrain is very different from that of the mid-ocean ridges, as judged, for example, from geological mapping in Iceland⁵. Kenya and Tanzania form part of an extensive Pre-Cambrian platform and Caenozoic volcanism is largely limited to a belt parallel to the Gregory Rift Valley which does not (except for two small volcanic centres) extend south of 3° 30' S. In the Western Rift Valley, the volcanism is limited to two elbows in fault direction and to the junction with the eastern rift at 9° S. Moreover, the volcanic rocks of East Africa are characteristically of alkaline type, whereas those of the mid-ocean ridges are almost entirely of the strongly contrasting tholeiitic type.

The floor of the rift valley itself is much cut by longitudinal faulting, but basement outcropping near the rift margins is not markedly cut by meridional dykes, as would be expected under an east-west tensional stress pattern. Girdler *et al.*¹ do not give the names of the volcanoes used in the computation, nor do they cite any isotope age data in addition to the stratigraphical ages given in the reports of the Geological Survey of Kenya. Many of the volcanoes have been active over quite long periods and, although it is known that the large majority of recent volcanoes and young fault scarps are associated with the relatively narrow rift valley zone, the volcanism had already started here in the late Miocene. On the other hand, activity on Mt Kenya and Kilimanjaro, some distance from the rift, continued into the Pleistocene⁶. Late Pleistocene craters are recorded⁷ in the Nyambeni Range north-east of Mt Kenya, and the craters of the Chyulu Range, north of Kilimanjaro, are presumed to be recent⁸. Both these ranges are over 100 km eastwards from the rift margin. It is therefore difficult to see how a general rule that volcanoes are older the farther they are from the rift axis can be established.

Whether the age of faults can be used as a measure of the rate of distension is also doubtful for the following reasons. The exact age of the rift faults in the area included in the computation of spreading rate is difficult to determine, although it is certainly well known that the youngest scarps are associated with the presently active rift valleys. The Gregory Rift Valley, however, fades out into block faulting to the south of 3° S and this factor must

complicate the assessment of data for even the most approximate calculation of a spreading rate because there is no central axis. Moreover, it is generally accepted that faults originating at least as far back as the Cretaceous have been reactivated at later dates up to recent. This characteristic of some of the faults of the East African rift system in Tanzania is therefore in sharp contrast to the situation on the mid-ocean ridges where the faulting at the axis affects only recent volcanic rocks. The geophysical information published by Girdler *et al.*¹ is of great value, and will help to explain, for example, the typical uparching of the rift belts which has been regarded as responsible for the rifting⁹; but before quantitative estimates of a spreading rate are accepted, much more must be known about the compatibility of the spreading hypothesis with continental crust geology of East Africa.

It has recently been suggested¹⁰ that certain dislocation zones, with local development of rift valleys^{11,12}, in the Guiana and West African shields lined up directly with the pre-drift fractures¹³. It is now widely accepted that these fractures later developed by ocean floor spreading into the basins of the present North Atlantic, South Atlantic and the connecting equatorial segment, which suggests that similar mantle structures underlie both continental and oceanic segments of these lineaments. It therefore seems that there is a similar type of lineament in both continental and oceanic lithosphere which, in the continental environment, may be affected by arching and consequent limited distension, or, in an oceanic environment, may be a locus of the rise of mantle material accompanied by ocean-floor spreading. Similar considerations apply to the Upper Rhinegraben lineament³ and have been suggested for the East African rift system¹⁴.

The East African rift lineament meets the spreading mid-ocean rifts of the Red Sea and Gulf of Aden in the Afar triangle, and it is tempting to seek evidence that the African rift is also spreading, particularly in view of the great volcanic outpourings in Ethiopia, although these are dominantly alkaline and not tholeiitic. But the rift valley and the belt of Caenozoic volcanics are on a scale which can be portrayed in detailed geological map sheets, so that verification of spreading, by reference to criteria now accumulated from the ocean floor rifts, should be possible by means of geological relationships¹⁵. The object of this communication is to indicate that the evidence for spreading at the rates postulated for the Kenya and Tanzania sectors by Girdler *et al.*¹ requires re-examination from the geological point of view because the phenomenon is fundamentally a geological one.

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Pleistocene Sea Levels possibly indicated by Buried Black Sediments in the Black Sea

MODERN surface sediments containing H_2S are often coloured black by fine grained, iron monosulphide minerals such as mackinawite, $Fe_{1+x}S$, and greigite, Fe_3S_4 . The black monosulphides are thermodynamically unstable, under H_2S -rich sedimentary conditions, relative to the disulphide pyrite, FeS_2 ^{1,2}, which is not a black pigments agent. As a result, the black colour normally disappears with depth during diagenetic transformation to pyrite^{2,3}. In some situations, however, such as in older buried layers of the deep water sediments of the Black Sea, the black monosulphides persist.

Volkov⁴ has shown that the black sediment of the Black Sea, when compared with the more common lighter grey sediment, is characterized by low concentrations of pyrite, lower total reduced sulphur, high concentrations of iron monosulphides, and very low dissolved H_2S in associated pore waters. He has suggested that this black sediment results from limited bacterial sulphate reduction during early diagenesis with insufficient hydrogen sulphide formed to enable complete transformation of black "FeS" to FeS_2 . Recent analyses confirm these conclusions (see Table 1). Methods of determination of each constituent have been described previously². Note that the total "reactive" iron content (pyrite iron plus HCl-(soluble iron) does not consistently differ between grey, pyritic sediment and black sediment. Thus the decrease in total reduced sulphur in the black layers must have been caused by a lower original production of H_2S by bacteria.

Limited H_2S production in anaerobic sediments and bottom waters is related primarily to (1) a low concentration of bacterially metabolizable organic matter, or (2) a low concentration of dissolved sulphate. Because the black layers of the Black Sea sediment are not distinctly different in organic carbon content from interbedded grey layers (see Table 1), barring such speculative factors as trace metal bacterial poisoning, the lowered H_2S production during deposition of the black layers must have been caused by a low concentration of dissolved sulphate in the overlying water. In a low sulphate situation, diffusion of sulphate into sediments may be too slow to provide enough sulphur for the complete diagenetic transformation of iron monosulphides to pyrite.

Table 1. MEASURED PARAMETERS FOR A CORE (1464-K) OF BLACK SEA SEDIMENT

Sampling depth range	Colour	Per cent sulphur as—		"Reactive" iron (per cent)	Organic carbon (per cent)
		$FeS + FeS_4$	FeS_2		
152-155 cm	Black	0.58	0.30	6.13	0.56
157-159	Black	0.74	0.22	7.10	0.58
162-164	Black	0.75	0.31	6.47	0.60
166-168	Grey	0.01	1.99	6.42	0.59
172-175	Grey	0.01	1.59	6.27	0.77
181-185	Grey	0.01	1.40	5.19	0.77
239-241	Grey	0.01	1.82	6.31	0.52
247-249	Black	0.33	0.30	5.18	0.59
259-262	Black	0.44	0.20	5.89	0.49
270-272	Black	0.58	0.44	5.76	0.71
279-281	Grey	0.01	2.52	7.06	0.59
284-287	Black	0.45	0.16	5.95	0.60
297-299	Grey	0.01	1.68	6.11	0.58
305-307	Black	0.67	0.30	6.46	0.68
329-331	Black	0.46	0.26	6.35	0.63
341-343	Grey	0.02	1.75	6.34	0.62
347-349	Black	0.39	0.46	6.75	0.73
362-364	Grey	0.02	1.56	5.60	0.87

The core was taken at 43°0' N, 35°5' E, water depth 2,179 m. Percentages are based on $CaCO_3$ -free acid soluble iron-free, dry weight. "Reactive" iron refers to the sum of pyrite iron and that soluble during boiling for 1 min in 12 N HCl.

Present day Black Sea water, which contains about 18 mmoles/l. dissolved sulphate, represents a mixture of fresh water derived from inflowing rivers with a high proportion of seawater derived from the Sea of Marmara by way of the Bosphorus. The sulphate is derived almost entirely from seawater, which contains about 28 mmoles/l. as compared with the rivers, which contain, on average, only 0.35 mmoles/l.⁶. Because of the high contribution

of seawater sulphate, black FeS minerals in the present deep water sediments are completely converted to pyrite⁵, resulting in grey sediment which is similar to the buried grey layers described in Table 1. During the Pleistocene, when the worldwide sea level was sufficiently lowered below the Bosphorus so that seawater inflow was excluded from the Black Sea, the constant influx of river water could have flushed out pre-existing sea salts, eventually resulting in a brackish or fresh bottom water low in dissolved sulphate. During these periods of maximum sea level lowering the black layers may have been deposited. Whenever the sea level rose high enough for seawater to spill over the Bosphorus sill, an influx of sulphate to the deep water would occur and black iron sulphides formed at the same time could be completely converted to pyrite resulting in grey sediment. Thus it is possible that sediment sections containing many black layers record periods of glacial maxima. (The simultaneous change in sea level and appearance of each black layer would not be expected because of complications arising from the interaction of seawater and freshwater in the Bosphorus and from time lags from rates of mixing of the two water types within the Black Sea itself.)

This suggestion must await detailed comparison of the age, thickness and frequency of black layers in undisturbed sections of deep Black Sea sediments with other indicators of Pleistocene sea level change. Preliminary examination of cores from the Black Sea deep basin suggests that the black layers are confined to an overall depth range of approximately the same age span as the last major glaciation. In addition, Markov *et al.*⁷ state that during the last major glaciation, the shallow waters of the Black Sea, as inferred from faunal evidence, were very dilute with salinities of less than 5 parts per thousand, which corresponds to less than 4 mmoles/l. dissolved sulphate. At certain times, the deep water was probably similarly dilute which enabled formation of the black layers.

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Photosensitized Oxidation of Ammonia by Singlet Oxygen in Aqueous Solution and in Seawater

THE various forms of inorganic nitrogen in seawater undergo complex transformations involving a variety of biological, chemical and photochemical processes which are important for protein synthesis and which determine the development of the marine ecosystem¹.

But the process involving nitrification of ammonia to nitrite and nitrate by chemical mechanisms is still not clearly understood.

Swallow² has recently shown that short lived chemical species such as hydrated electrons can play an important part in seawater processes. Singlet oxygen is another

short lived species the importance of which has recently been demonstrated³, and we have examined its effect on aqueous ammonia and how it could be produced in seawater.

Singlet oxygen was produced by dye photosensitization of aerated solutions of eosin, rose bengal, methylene blue or riboflavin. When a solution containing ammonia, 10^{-2} M, and a dye, 10^{-4} M, is irradiated with visible light which is absorbed by the dye in the presence of air, nitrite and nitrates are produced but none appear if the dye or ammonia is absent under the same illumination conditions. (It has been shown that in some conditions N_2 can be oxidized to NO_2^- by singlet oxygen⁸.) The nitrite ions are determined by colorimetry using Bendschneider⁴ and Robinson's method⁴ and the nitrate ions by Taras's method⁵ using phenol disulphuric acid. Experiments were also carried out in seawater to which ammonia and dyes were added. Identical results were obtained.

The nitrite and nitrate yields are small. Exposing the solutions described here, adjusted to pH 8.5, to a 300 W tungsten lamp in a Pyrex cell, gives about 10^{-5} M of both ions after 2 h of illumination. The yields increase with pH, which indicates that the oxidized species is NH_3 rather than NH_4^+ .

Dyes in aqueous solution are destroyed by light even in the absence of added solutes. The decomposition is even more rapid in the presence of buffer mixtures such as phosphates, carbonates and borates, whether in oxygenated or in unoxxygenated solutions. Because oxidation of leuco dyes by oxygen in water gives peroxides⁶, we have checked that this process is not responsible for the oxidation of ammonia.

We also prepared singlet oxygen by another procedure, using an apparatus designed by Douzou and Balny⁷. Hydrogen peroxide and sodium hypochlorite are allowed to react in one compartment, producing singlet oxygen which is carried by a stream of nitrogen to another compartment 3 cm away where it is bubbled through an ammoniacal solution. Nitrite and nitrates are produced, but again in small quantities, and the yields also increase with the pH of the solution. We therefore conclude that singlet oxygen, $^1\Delta$, is able to oxidize ammonia in aqueous solution.

Numerous aromatic compounds in seawater can photosensitize the production of singlet oxygen. Among the substances which absorb in the visible range, riboflavin seems to be the most promising species, although its concentration is very small. With substances which absorb in the near ultraviolet, the photosensitive process competes with direct photo-oxidation of ammonia⁸. We have found that this latter process becomes very important with light of wavelength below 320 nm.

It would be interesting to estimate the amount of nitrification in seawater from the photosensitized mechanism, and to extend this finding to other media, such as waste water containing ammonia and organic compounds.

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New High Temperature Carbon Fibre Composite

We have prepared in this laboratory carbon composites reinforced with carbon fibres which have flexural strengths of 120,000 to 140,000 pounds per square inch at room temperature and 125,000 to 150,000 pounds per square inch at 1,500° C (in helium). The composites are reinforced unidirectionally with carbon fibres which make up 75-80 per cent of the total by weight. Densities range from 1.4 to 1.64 g/ml.

The composites are prepared by impregnating Morganite I carbon fibre bundles with one of several possible resins in solution, evaporating off the solvent in vacuum and curing the sample in a mould under heat and pressure. The resulting carbon fibre reinforced polymer composite is then heated in vacuum at high temperature (700°-1,000° C) to carbonize and volatilize the polymer. This treatment is followed by the deposition of pyrolytic carbon within the resulting porous structure using a hydrocarbon gas at high temperature in carefully controlled conditions. By this technique it is possible to replace all or more of the weight lost on pyrolysis with pyrolytic carbon. This can be done in a few hours for small samples made from polymers giving a high char yield, but better results are obtained by extending the deposition of carbon to longer periods.

The flexural strengths were obtained on samples 1.5 inches long, 85 mils wide and 53 mils thick with a span of 1.2 inches and a loading nose with a radius of 3/8 inch moving at 0.05 inch/min. The stress-strain curves were straight and their slope yielded an elastic modulus of 20-30 million pounds per square inch for the composites. All of the failures were caused by crushing on the compression side. Interlaminar shears run on the same sized samples with a span of 0.3 inch and a loading nose radius of 1/16 inch gave values from 2,000-4,500 pounds per square inch at room temperature.

The impact strength of these composites is comparable with the impact strength for epoxy matrix composites made with the same carbon fibre. This high strength, however, must be set against the poor resistance to oxidation of these composites at high temperatures.

Ageing at 500° C in air for eight hours reduced the interlaminar shear strength to less than 50 per cent of the original value. Methods of protecting the composites from oxidation and improving interlaminar shear are now being investigated.

Fuller details of this procedure will be made available after patent applications now pending have been granted.

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Polyethylene Spherulites

THERE has been some controversy about the report that polyethylene does not produce spherulites resolvable with a light microscope¹⁻³ but instead produces a complex birefringent mass made up of units less than 1 μ m in diameter (see Figs. 1 and 2 of ref. 1). Previous reports that polyethylene readily produces spherulites, 0.1 to 1.0 mm in diameter^{4,5}, have been attributed to the use of excessive pre-heat treatment above 250° which degrades the polymer, produces excessive thinning of the sample films and destroys nuclei. Organ (private communication), however, considers that the lack of resolution is entirely due to

sample thickness effects such that the large amounts of birefringent material present cloud the contours of the spherulites, which can be clearly observed with samples of 10 μm thick or less.

Thin films of 'Rigidex' polyethylene, 10 μm thick, were observed to exhibit well defined spherulites, 2-5 μm in diameter, and on subsequent melting and re-crystallization the granular birefringent patterns observed before¹⁻³ were reproduced. On melting, the thin films were observed to collapse into very much thicker globules. Similar studies with Marlex-60 samples were inconclusive, for the thin films (4 μm thick) exhibited no well defined spherulites.

In order to define the structural units present in the Marlex-60 the surfaces of bulk and thin film samples were examined with a 'Stereoscan' scanning electron microscope. In each sample, crystallized both in bulk and in thin films, the same structural unit of predeterminedly nucleated spherulites was observed. All the diameters (0.2-1.5 μm) were consistent with those estimated in ref. 1.

Table 1. POLYETHYLENE-MARLEX-60 SAMPLES

Fraction	Weight average Molecular weight	Dispersity Mw/Mn	Texture
A	142,000	7.4	Very coarse
B	41,200	1.6	Fine
C	98,900	3.1	Coarse
D	165,400	2.1	Fine

Two extreme morphologies of the spherulites were observed (see Figs. 1 and 2), (a) of equally sized close-packed spheres with a fine structure of concentric rings and (b) of equally sized spherical bundles of coarse fibres. Despite the complexity of the second morphology, spherical contours can be seen clearly at low magnification and spherulitic growth was obviously present. Other samples of Marlex-60 exhibited this coarse texture to different degrees, which appeared to correlate closely with the dispersity of the samples (see Table 1). This is in agreement with "impurity" segregation theory of spherulite crystallization⁶ in which the less crystallizable materials of lower molecular weight are rejected by the growing face; growth then develops from fibres which grow branch laterally⁷.

The morphologies also correlated closely with those observed in the larger spherulites observed in polyethylene^{4,5,8} and indeed the spacings between the fine structure of concentric rings (0.5-5.0 μm) are consistent. Because there is no difference in morphology, differences between the polyethylene samples lie in their thickness and also in the density of primary nuclei present. Bulk crystallized polyethylene clearly has a spherulitic texture.

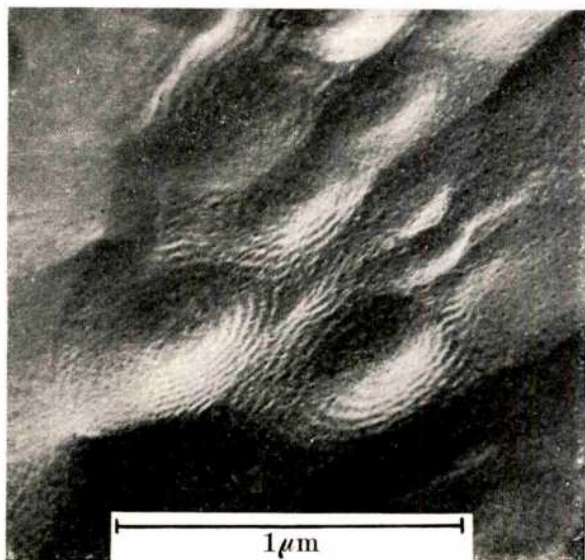


Fig. 1. Sample D crystallized at 124.0° C ($\times 4,500$).

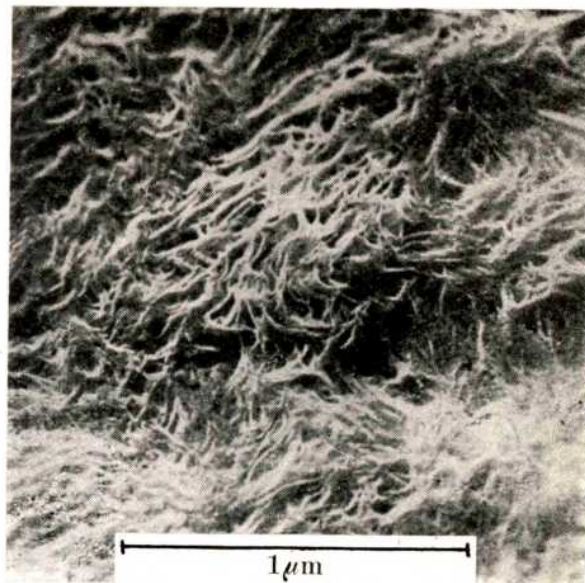


Fig. 2. Sample A crystallized at 124.0° C ($\times 4,500$).

It has been suggested that preferential electron etching on the polymer surface of low molecular weight regions, shown in Fig. 2, may have high-lighted the spherulitic structure. This, however, in no way alters our conclusions.

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Ionization, Attachment and Breakdown in SF₆

SULPHUR hexafluoride is widely used as an insulator in many electrical devices, but little is known of its fundamental electrical properties, particularly at voltages above about 100 kV. As the first part of an investigation on the growth of pre-breakdown ionization in SF₆, we have carried out measurements from which values of the apparent primary ionization coefficient α' have been determined, at pressures in the range from 50 to 400 torr and for voltages up to about 200 kV. We report here a dependence of the coefficient α' on gas pressure, not previously observed in SF₆, which is significant to the insulating properties of the gas and which can be related to the fundamental collision processes occurring.

At low pressures ($< 10^{-5}$ torr), previous single collision experiments^{1,2} indicated that the main negative ion

species formed in SF_6 by electron collisions were SF_6^- and SF_5^- . In addition, the negative ion currents were observed to depend on pressure rather than on the square of the pressure, which, in the case of SF_6^- , indicated that the ions were in an excited state. Subsequent experiments³⁻⁵ showed that the lifetime to autodetachment of the excited ion $(\text{SF}_6^-)^*$ is very long, $>10^{-6}$ s.

At relatively high pressures (≥ 10 torr), experiments⁶ on the movement of electron swarms in SF_6 have shown that, at these pressures also, both SF_5^- and SF_6^- ions are formed, the relative abundance being dependent on the ratio E/p_{20} (where E is the electric field and p_{20} the pressure reduced to 20° C). At these pressures the $(\text{SF}_6^-)^*$ ions formed in electron collisions with gas molecules make a large number of collisions during their lifetime, so that it is likely that the observed SF_6^- ions are collision stabilized. If this is the case, the attachment coefficient would be expected to be dependent on p_{20}^2 . Measurements^{6,7} of attachment coefficients to date, however, do not seem to indicate such a dependence over the pressure range from 5 to 100 torr. In order to investigate further this apparent contradiction, it seemed worth while to carry out further pre-breakdown ionization measurements over an extended range of gas pressures up to 400 torr.

The apparatus and experimental procedures used were basically the same as those described previously⁸, only slight modifications to the equipment (such as placing activated alumina traps in the pressurizing line and ionization chamber, for example) being necessary for the work with SF_6 .

Measurements were made of the ionization currents as a function of electrode separation at one value of $E/p_{20} = 118 \text{ V cm}^{-1} \text{ torr}^{-1}$. The ionization chamber was filled to a pressure of about 400 torr with SF_6 from a high pressure gas cylinder supplied by Laporte Industries Limited, the quoted impurities being 0.05 per cent of air, 0.05 per cent of CF_4 and 15 p.p.m. of water vapour. Measurements were obtained at this pressure and also for the same sample after reducing the pressure in seven steps, each of 50 torr. A least squares procedure for curve fitting⁹ was used to analyse the measurements in terms of the equation

$$\frac{I}{I_0} = C \left\{ \frac{\alpha' + a}{\alpha'} e^{\alpha'd} - \frac{a}{\alpha'} \right\} \quad (1)$$

where I is the ionization current at an electrode separation d ; $I_0 = I_0/C$, where I_0 is the initial photo-electric current and C is a constant; the coefficient α' is the apparent ionization coefficient given by $\alpha - a$, where α is the Townsend primary ionization coefficient and a the attachment coefficient.

Because I is exponentially dependent on $\alpha'd$, the coefficient which is determined with greatest accuracy in this type of experiment is α' . The errors in the determination of a are much larger, so that any dependence of a

on the square of the pressure is, in fact, most likely to be detected through its effect on α' . The values of α'/p_{20} determined in our investigation are given as a function of pressure in Fig. 1, and it can be seen that α'/p_{20} decreases as the pressure increases, the variation being well outside the experimental error, which is about ± 2 per cent. This variation indicates that in SF_6 , in the conditions of the investigation, one or more processes are operative which depend on the square of the pressure. Bearing in mind the previously determined existence of SF_6^- ions in swarm conditions, it seems likely that this process is collisional stabilization of $(\text{SF}_6^-)^*$ ions, which are produced in the swarm by electron collision.

The work is now being extended to other values of E/p_{20} and to an investigation of whether there are departures from Paschen's law in SF_6 as implied by these results.

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Rate Equations in Thermogravimetry

MacCallum and Tanner¹ have argued persuasively for modifying the rate equations used in thermogravimetry, but their conclusions are incorrect. There is no fundamental error of the kind they suggest in the usually accepted procedures and some other reason must be found for discrepancies in derived kinetic parameters. In the present context the point at issue has been dealt with by Kissinger², as follows.

"When the temperature is changing with time, the reaction rate is

$$\frac{dx}{dt} = \left(\frac{\partial x}{\partial t} \right)_T + \left(\frac{\partial x}{\partial T} \right)_t \cdot \frac{dT}{dt}$$

The rate of change of x with temperature, with the time coordinate fixed, $(\partial x / \partial T)_t$ is zero, because fixing the time, also fixes the number and position of the particles constituting the system. The only effect of an instantaneous change in temperature is in the velocity of thermal motion of the particles."

Thus dx/dt always equals $(\partial x / \partial t)_T$, so that the chemical reaction rate is given by the slope of a plot of x against t whether the temperature is varying or not.

It is possible that confusion arises when chemical change is thought of as contributing to the state of a system, rather than as a means whereby states are transformed. The situation is analogous to that of the arrow in flight³, considered by Zeno; although the arrow is in motion, at any instant it is at rest. Similarly the state of a system, reacting chemically can be defined at any instant of time, without reference to change and provided we make an exception of processes so fast that the identity of chemical

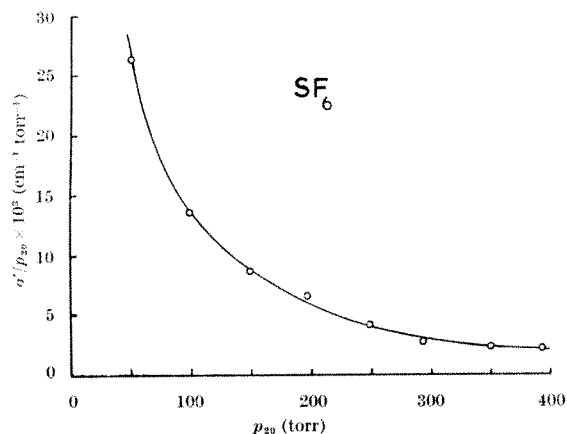


Fig. 1. α'/p_{20} , p_{20} at $E/p_{20} = 118 \text{ V cm}^{-1} \text{ torr}^{-1}$.

species and the meaning of temperature are in doubt, this definition can be put in ordinary thermodynamic terms. In thermal analysis change is so slow that temperature has its usual thermodynamic significance, so that $(\partial x/\partial T)_t$ is effectively zero in the experiments considered by MacCallum and Tanner.

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Rate of Hydrogen Dissociation at a Hot Tungsten Surface

THE atomization of molecular hydrogen at a hot tungsten surface has been studied extensively, some of the discussion being concerned with the correctness or otherwise of Langmuir's¹ early work. Moore and Unterwald² have summarized the various investigations up to 1964. In particular, it has often been stated that Langmuir's rates were "too low by factors which were all greater than 200", the quoted assertion being first made by Bryce³ but often repeated since then (see for example refs. 3-6).

Langmuir used two techniques to measure the atomization rate. The first relates the rate of heat loss from a tungsten filament in a hydrogen atmosphere to the atomization rate, and is the more reliable². In the second, the rate of removal of hydrogen by an "atom trap" is assumed to be equal to the rate of production of atoms by the hot filament. Until mass spectrometric methods were introduced, only the latter of these two techniques was used by other workers, and it is their results which are said to conflict with Langmuir's work. Recently, Roberts and Young⁷, in studies of the kinetics of the interaction of hydrogen atoms with lead films, concluded that the incorporation of hydrogen into the lead lattice is activated, and is the slow step. At 78 K the rate of incorporation is a factor of nearly 100 slower than that observed with the film at 300 K. This result, like the earlier one of Anderson and Ritchie⁸ with sodium films, implies that atom traps can vary in their trapping efficiency and in general are not expected to be perfect, still less to give rates much in excess⁶ of those derived from heat loss measurements. We have accordingly examined the evidence for the alleged discrepancy (the factor of > 200 by which Langmuir's rates were alleged to be too low) and conclude that it is without justification.

The most careful of the atom trap studies is that of Brennan and Fletcher⁴, who obtained the following expression for the atomization rate n (atoms s⁻¹ (cm² of W)⁻¹)

$$n = 18 \times 10^{24} \sqrt{p} \exp(-52,600/RT)$$

where p is the hydrogen pressure in torr.

Strictly speaking, this result holds only up to 1,400 K, and extrapolation to higher temperatures, necessary for comparison with Langmuir's heat loss data, would not be justified if the hydrogen pressure were less than 10⁻³ torr (ref. 4). Consider first Langmuir's heat loss data (over and above that caused by radiation and convection) for a hydrogen pressure of 1.5 × 10⁻² torr and in the temperature range 1,800-2,900 K (ref. 1, page 435). The atomization rate can be computed from these data by equation 17 of ref. 1, page 427. For example, at 1,800 and 2,100 K, the rate is found to be 8.4 × 10¹⁷ and 5.7 × 10¹⁸ atoms s⁻¹ (cm² of W)⁻¹ respectively, the requisite heats

of dissociation being taken from Stull and Sinke⁵. The corresponding values from Brennan and Fletcher's equation (4) are 9.1 × 10¹⁷ and 7.4 × 10¹⁸ atoms s⁻¹ (cm² of W)⁻¹, which are in good agreement with Langmuir's values. Even Bryce's data, which are considered to be in error from poor vacuum techniques, yield rates at 1,800 K and 2,100 K of 1.0 × 10¹⁸ and 6.4 × 10¹⁸ atoms s⁻¹ (cm² of W)⁻¹, again in close agreement with Langmuir. The comparisons are almost as good if made at a hydrogen pressure of 1.1 torr, for which Langmuir also provides heat loss data.

Langmuir himself did not rate his second technique, in which atoms were trapped on a cooled glass surface, as highly as the first (ref. 1, page 452). Nevertheless, the results obtained from it compare favourably with those obtained by Bryce, who used the much more efficient MoO₃ trap. According to Langmuir's experiment 160 (ref. 1, page 541), the rate of hydrogen uptake on the cooled glass surface of the containing vessel was 1.4 mm³ min⁻¹ (cm² of W)⁻¹ at a hydrogen pressure of 1.5 × 10⁻² torr and a tungsten temperature of 1,200 K. This corresponds to a rate of 1.3 × 10¹⁵ atoms s⁻¹ (cm² of W)⁻¹ and compares favourably with the data of Bryce which yield a rate of 1.9 × 10¹⁵ atoms s⁻¹ (cm² of W)⁻¹.

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NMR Relaxation in Heterogeneous Systems

THERE has recently been some discussion^{1,2} of the difficulties of interpreting nuclear magnetic resonance line broadening in complex heterogeneous suspensions. The discussion has been concerned with hindered rotation of solvent species, and it has been confined to proton magnetic relaxation. We report here a study of the relaxation phenomenon in a well defined physicochemical system and contrast the results for proton and deuteron relaxation.

It is well known to NMR spectroscopists that particulate matter in a sample tube causes line broadening. The exchange of solvent species between the bulk phase average local magnetic field and that which exists near suspended matter is sufficient to cause broadening, and no reference to ordering or disordering of solvent is necessary to interpret it. The parameter necessary for interpretation in terms of disordering is the intra-term³ for the spin-lattice relaxation time (T_1) which is obtained, only with great difficulty, from proton relaxation, not to mention line-width (or free induction decay), measurements.

A model system with quite well defined surface to volume ratios may be constructed by packing spheres of known radius in an NMR sample tube with the excess volume filled with water. Glass spheres with quite uniform diameters down to 15 μm are now commercially

available (Microbeads Div., Cataphote Corp.). In addition, they are available with a monomolecular surface coating of a silicone compound (dimethylsilane). Under microscopic examination these spheres, made of optical crown glass, are very smooth and have a narrow distribution of diameters within the manufacturers' specified characteristics.

Fig. 1 shows the results of proton T_1 (null point) and T_2 (Carr-Purcell-Meiboom-Gill) measurements as a function of radius for coated and uncoated spheres at 30 MHz and 26.5° C. The samples were carefully packed to constant and equal total volumes.

Fig. 2 shows similar results for T_1 (adiabatic fast passage⁴) for deuterium in $^2\text{H}_2\text{O}$ in analogously prepared samples.

Two conclusions are immediately clear from these figures. First, T_2 measurements, properly carried out by the spin-echo Carr-Purcell method, are not sufficient for determining rotational reorientation times for protons since $T_2 \ll T_1$ in all the systems. Second, proton measurements in such systems cannot be used to derive an intra-term, and therefore rotational reorientation times, because the change in proton T_1 as the surface-to-volume ratio rises is very much larger than that for deuterium T_1 . Clearly, the proton inter-term has become very important.

The explanation for the above is not difficult to find, and has been discussed quantitatively for protons at much lower surface-to-volume ratios than described here⁵. Essentially, in heterogeneous systems such as this, the effective self-diffusion coefficient of the water molecules is greatly reduced because of mechanical trapping of molecules in surface crevices and between surfaces. Thus, for protons, the inter-term, which is of the same magnitude as the intra-term at low surface-to-volume ratios, becomes very large. The effects observed in proton T_1 measurements in such heterogeneous suspensions are dominated by the inter-term and cannot be interpreted in terms of solvent rotational mobility effects. This conclusion holds true in studies of biological and non-biological systems. It is not true in $^2\text{H}_2\text{O}$ systems where the inter-term is negligible⁶.

Fig. 2 shows that there are changes in rotational mobility at such surfaces, as described here, and that the mobility changes when the nature of the surface changes. The magnitude of the changes is, however, very much smaller than might be inferred from properly interpreted proton relaxation experiments.

Similar remarks apply to all experiments on heterogeneous and colloidal biophysical systems because they depend on large surface-to-volume ratios.

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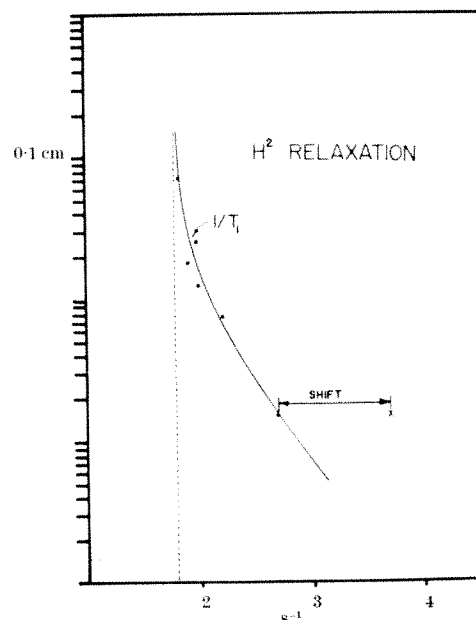


Fig. 2. Deuteron relaxation rates and diameter of glass beads for suspensions of unispheres in $^2\text{H}_2\text{O}$. "Shift" as in Fig. 1.

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Electronic Conduction across a Black Lipid Membrane

BLACK lipid membranes (BLM) have been found to be useful and realistic models for biological membranes¹, showing selective ionic^{2,3} and proton^{4,5} permeabilities, differential negative conductance^{6,7}, the photovoltaic effect⁸, excitability⁹, ability to support active transport¹⁰ and the capacity to interact with leucocytic granules¹¹. Biological membranes may also function in the transport of electrons between redox carriers on opposite sides of the membrane: Mitchell¹² has advocated the coupling of such electron transfer with transmembrane proton translocation as an essential aspect of energy conservation in the redox process of the respiratory chain in mitochondrial and photosynthetic systems. We describe here a partial model for this process in which electronic charge transfer occurs across a BLM.

Two considerations suggest that a combination of iodide and iodine may serve as a mediator for electron transfer across thin membranes. First, these species in combination increase the conductance of certain BLM by several orders of magnitude^{13,14}. This fact evidently reflects the tendency of these species to form triiodide, I_3^- , and polyiodides, I_n^- , which, because of extensive charge delocalization, have substantial affinity for lipid membranes. Second, poly-

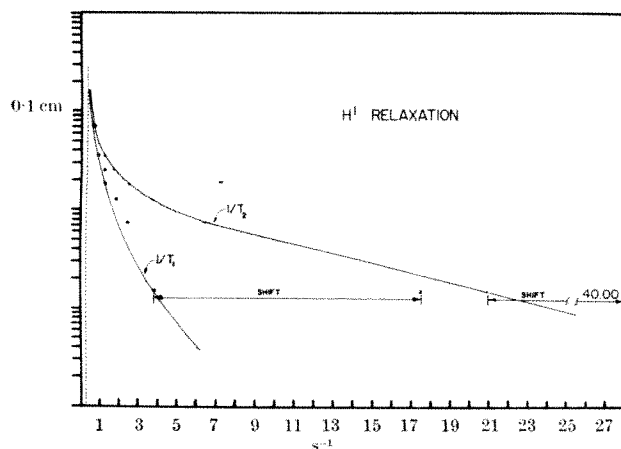


Fig. 1. Proton relaxation rates and diameter of glass beads for suspensions of unispheres in H_2O . "Shift" refers to change between uncoated (left side) and coated (right side) beads of the same diameter.

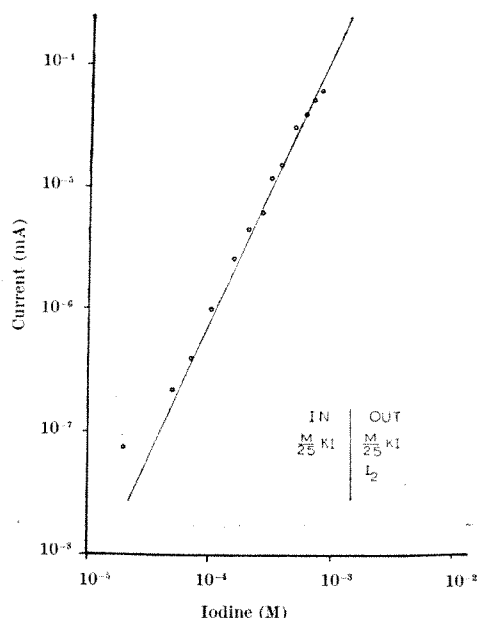


Fig. 1. Zero volt current across BLM as a function of iodine concentration on one side. Iodine solution in M/25 KI, together with suitable buffer and other additives, gave the required iodine concentration. The direction of electron flow was towards the side containing iodine.

iodides have unfilled *d* orbitals at low energy which might be used in electron conductance. Moreover, iodide, iodine and polyiodides take part in several redox reactions, so that these species may participate both as components and mediators in such reactions.

In one case, the I^- - I_2 induced conductivity increase across a BLM has been shown to result from ionic, not electronic, conduction¹³. This observation does not, of course, eliminate the possibility of electronic conductance, particularly when a BLM of abnormally high resistance to ionic conduction is used.

The BLM studied here is constructed from 1 per cent oxidized cholesterol¹⁵, octane-dodecane and didodecyl phosphate as previously described¹⁰, and is supported on a 1 mm diameter hole in a septum of a two-compartment 'Teflon' chamber. Electrical measurements were made as previously described¹⁰. In all the experiments reported here, the membrane was bathed on both sides by aqueous solutions containing 0.10 M NaCl, 0.01 M KCl, 0.01 M MgCl, and 0.02 M *tris*(hydroxymethyl)aminomethane, pH 7.4. All measurements were made at 25°C. This membrane has a resistance of about $\sim 10^{10}$ – $10^{11} \Omega \text{ cm}^{-2}$.

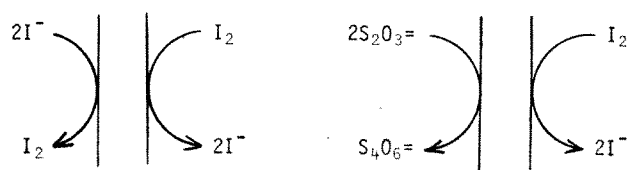
In accordance with previous observations^{13,14}, addition of I_2 ($5 \times 10^{-4} \text{ M}$) and KI (0.025–0.04 M) to the solutions bathing the membrane reduces the resistance dramatically, to $2.6 \times 10^3 \Omega$ in this case. A plot of diffusion potential against the logarithm of the ratio of iodide ion on the two sides of the membrane is linear, with a slope of 60 mV per concentration decade when the ratio is 10 or less¹³. For higher ratios, the plot becomes non-linear with a continuously decreasing slope.

Addition of iodine to the compartment on one side of the BLM under conditions in which 0.04 M KI is present on both sides causes a net flow of positive current across the BLM from the side containing the iodide to the other. As shown in Fig. 1, a plot of the logarithm of net zero volt current flow across the membrane against the logarithm of iodine concentration is nearly linear: the corresponding non-logarithmic plot is sigmoidal with appreciable current observed above $5 \times 10^{-5} \text{ M}$ iodine and saturation approached near $4 \times 10^{-4} \text{ M}$. In these conditions, only 1–3 mV of open circuit voltage developed.

Similarly, addition of thiosulphate ($\sim 1 \times 10^{-4} \text{ M}$) to one side of the membrane when the other compartment contains an excess of iodine elicits a net flow of positive

current across the BLM from the side containing the iodine to the other. This current flow is accompanied by an increase in membrane resistance, perhaps as a consequence of diminution of iodine concentration.

The direction of current flow in these systems establishes that the conductance across the membrane is electronic; ionic conductance would require the current to flow in the opposite direction. Both the sign and magnitude of the diffusion potential require that the conductance be due to anionic species. We must therefore have redox reactions across the membrane, mediated by polyiodide ions spanning it, which may be schematically visualized in the following way:



These two redox reactions occur by way of electron transfer across the BLM without appreciable transfer of any iodine-containing species. This conclusion is based on the following observations: (i) No flux of ^{131}I across the BLM could be detected over 30 min in conditions of diffusion, under the influence of a favourable electrical field, or under the influence of iodine or thiosulphate gradients, even though the resistance of the BLM in the presence of iodine and iodide (2.6 – $3.4 \times 10^3 \Omega$) is only 3–5 times greater than that for the same system without a BLM, $0.8 \times 10^3 \Omega$. In the absence of a BLM, ^{131}I is equilibrated between the two compartments with a half-life of about 3 min. (ii) Moreover, under conditions of passage of $1 \mu\text{A}$ (10^{12} charges/s) across the BLM for 45 min, not more than 0.002 per cent of ^{131}I initially added to one side

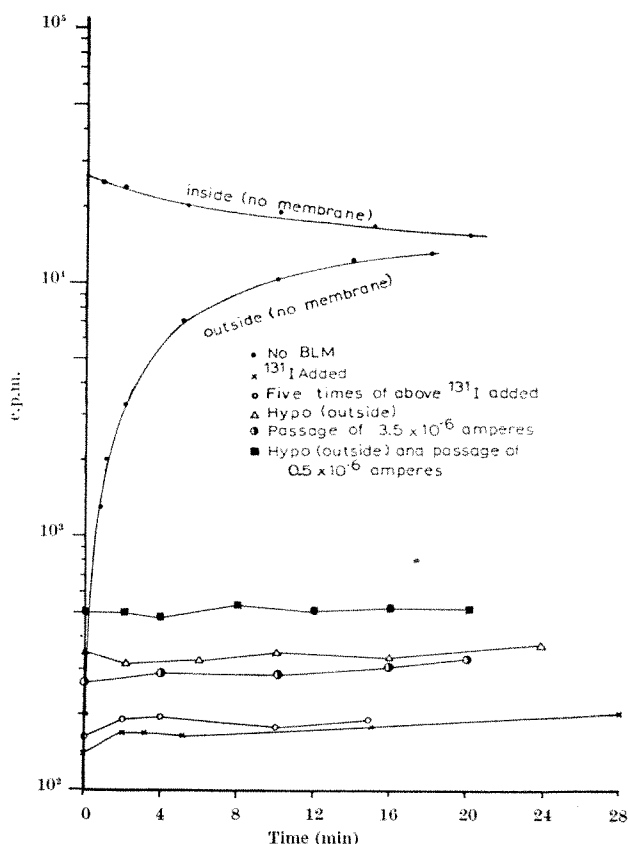


Fig. 2. Flux of ^{131}I across BLM in various conditions. Concentration of KI in both compartments was kept at 5 mM in all experiments.

of the membrane is transported to the other, although simple calculations indicate that at least 10 times this amount, even ignoring simple diffusion, ought to have been transported if the conductance were ionic. These results are summarized in Fig. 2. In an effort to understand something about the nature of the process in the BLM which accounts for the electronic conductance, several aspects of BLM behaviour in the presence of iodine-containing species are being investigated; results will be published soon. Overall, these observations are consistent with polyiodide-mediated electron transfer across the BLM.

Qualitatively similar results to those just described have been obtained using a lecithin:cholesterol:tetradecane and a lecithin:tetradecane BLM. The corresponding isotopic flux experiments have not been performed with these membranes.

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BIOLOGICAL SCIENCES

Chromosome Breakage by Deoxyribonuclease

THE possibility that endogenous deoxyribonuclease might play a part in inducing chromatid breakage was suggested by our experiments describing a high incidence of chromosome breaks and rearrangements in human diploid cell strains after selective damage to lysosomes¹. We describe here a similar effect when exogenous deoxyribonuclease is introduced into cells in the presence of hypertonic MgSO_4 . This is a modification of the method used by Wecker² in assays of the infectivity of extracted viral nucleic acid.

Pancreatic deoxyribonuclease, recrystallized once, was purchased either from Worthington Biochemicals (approx. 2,400 kU) or from Sigma, electrophoretically purified (approx. 1,900 kU). The enzyme was dissolved in 1.5 M MgSO_4 at a final concentration of 1,200 kU/ml. and stored at -20°C . Thermal inactivation was effected by standing the solution of enzyme in MgSO_4 in a boiling water bath for 20 min. Highly purified lysosomal deoxyribonuclease, extracted from hog spleen³, was dialysed overnight against

1.5 M MgSO_4 before use (final concentration = 330 U/ml.). *Escherichia coli* endonuclease and exonuclease (20 U/ml.) were purified as described⁴ elsewhere. The double-stranded polyribonucleotide, poly A-poly U, which is a competitive inhibitor of lysosomal deoxyribonuclease⁵, was dialysed against 1.5 M MgSO_4 , dispersed by ultrasonic vibration and mixed with lysosomal deoxyribonuclease immediately before addition to the cell sheet. Spectrophotometric studies confirmed that both enzyme and inhibitor were present in the solution. Pancreatic deoxyribonuclease acts by splitting one or another of the two strands of the DNA molecule. The kinetics show "double-hit" cleavage of the duplex. By contrast the lysosomal enzyme attacks double-stranded DNA by splitting both strands simultaneously at the same level. The kinetics show "single-hit" breakage of the double strand. *E. coli* endonuclease preferentially attacks native double-stranded DNA with single-hit kinetics of cleavage of the DNA duplex. *E. coli* exonuclease cleaves mononucleotides from the 3' hydroxyl ends of a single strand of DNA.

In most experiments, the WI 38 strain of diploid human embryonic lung cells⁶ was used. Culture methods were described previously¹. In one experiment adult human diploid fibroblast cultures, grown in the same conditions, were used.

For the hypertonic treatment, nucleases were introduced into the cells approximately 20 h after trypsinization by the following method. Medium was replaced with 5 ml. Mg^{2+} -saline (0.02 M MgSO_4 in Ca^{2+} -free phosphate buffered saline, Dulbecco 'A' medium, pH 7.0); the cells were incubated in this for 15–20 min at 37°C and rinsed with a fresh solution of Mg^{2+} -saline. After being drained thoroughly, the cell sheet was treated with 1 ml. of 1.5 M MgSO_4 (pH 5.5) containing the required concentration of enzyme and rocked for 15 min at room temperature. The cells were rinsed three times in growth medium before being returned to normal culture conditions. In some control groups the culture medium only was changed; others were treated with Mg^{2+} -saline and 1.5 M MgSO_4 , omitting the enzyme.

In some experiments hydrocortisone (Merck) was added to the culture medium at a final concentration of 500 $\mu\text{g}/\text{ml}$. for 5 h before enzyme treatment, and 250 $\mu\text{g}/\text{ml}$. after treatment until the time of fixation.

A suitable time after exposure to enzyme (23–27 h) cultures were treated with colchicine (0.8 $\mu\text{g}/\text{ml}$.) for 3–5 h. The cells were suspended by trypsinization, treated with hypotonic solution (0.075 M KCl)⁷, fixed rapidly in methanol-acetic acid (3:1), spread on chilled slides, air-dried and stained with 1 per cent acetic-orcein. Metaphases suitable for chromosome examination were selected under low magnification and studied under oil immersion for any abnormalities. These were classified as (a) chromatid breaks, showing non-alignment or axial displacement of the broken ends, and (b) chromatid gaps, which included non-staining regions or small breaks in one chromatid; secondary constrictions were not scored. The figures in the tables are the percentages of cells examined with one or more chromatid breaks. Chromosome fragments and structural abnormalities (for example, dicentric and acentric) were rare and are not included in the tables.

As shown in Table 1, the incidence of chromatid breaks in human diploid cells treated with either pancreatic or lysosomal deoxyribonuclease was significantly higher than in control cells either untreated or exposed to Mg^{2+} -saline and MgSO_4 in the absence of enzymes. In cells exposed to pancreatic deoxyribonuclease inactivated by heating, or to the lysosomal enzyme and an inhibitor (poly A-poly U), the incidence of chromosome damage was comparable with that in the controls. Addition of hydrocortisone to the deoxyribonuclease treated cultures did not reduce the incidence of chromosome damage (Table 2). Because hydrocortisone is a well-known

stabilizer of lysosomal membranes⁸ this result makes it unlikely that endogenous lysosomal enzymes are being released by the deoxyribonuclease treatment. Further studies with an endonuclease from *E. coli* produced a relatively high incidence of chromatid breaks, whereas the exonuclease had no effect (Table 3).

The hypertonic MgSO_4 technique, originally used for introducing infectious viral nucleic acid into mammalian cells², has proved effective in facilitating the entry of deoxyribonuclease into cell monolayers. In spite of the high molarity of the MgSO_4 , this treatment alone had no observable effect on chromosomes, and the cells—although vacuolated immediately after treatment—seemed to recover completely and grow normally, in keeping with previous observations of Holland⁹, who used 2 M MgSO_4 on HeLa cells.

Table 1. INCIDENCE OF CHROMATID BREAKS IN WI 38 CELLS AFTER TREATMENT WITH VARIOUS NUCLEASES

Treatment	No. cells examined	Percentage cells with chromatid breaks
Untreated control	519	3.5
MgSO_4 rinsed control	119	2.8
Pancreatic deoxyribonuclease	302	11.9*
Lysosomal deoxyribonuclease	377	10.4*
Lysosomal deoxyribonuclease + poly A-poly U	169	4.7
Pancreatic deoxyribonuclease (heated)	120	2.5

* $P < 0.001$ analysed by a $2 \times 2 \chi^2$ test.

Table 2. EFFECT OF HYDROCORTISONE ON THE INDUCTION OF BREAKAGE IN WI 38 CELLS BY PANCREATIC DEOXYRIBONUCLEASE (0.05 PER CENT)

Treatment	No. cells examined	Percentage cells with chromatid breaks
Untreated control	267	4.9
Control + hydrocortisone	94	2.1
Pancreatic deoxyribonuclease	361	10.8*
Pancreatic deoxyribonuclease + hydrocortisone	78	12.8*

* $P < 0.001$ analysed by a $2 \times 2 \chi^2$ test.

Table 3. EFFECTS OF *E. coli* DEOXYRIBONUCLEASES ON ADULT SKIN FIBROBLAST CULTURES

Treatment	No. cells examined	Percentage cells with chromatid breaks
Untreated control	122	2.5
Endonuclease	78	9.0
Exonuclease	73	1.4

Several factors indicate that the effects described here are attributable to exogenous enzyme rather than to activation of lysosomal or other endogenous enzyme systems. One is the uniformity of the response after introduction of the three types of endonucleases all of which attack intact strands of DNA in chromosomes, contrasted with the lack of effect with exonuclease. Second, when the enzyme was inactivated or when a selective inhibitor of the nuclease was used, the effect was absent. Third, treatment of cells with hydrocortisone did not reduce the chromatid breakage. A fourth point is that the extent of the chromatid damage is proportional to the concentration of deoxyribonuclease introduced (unpublished).

Thus when various endonucleases are introduced into mammalian cells they are not degraded in the host cell cytoplasm nor is their passage prevented by the nuclear membrane; they can enter the nucleus in an enzymically active form and produce chromosomal damage. Robbins *et al.*¹⁰ describe electron microscopical observations of the response of HeLa cells to hypertonic solutions. They report clumping of the chromatin around the nuclear periphery and partial disintegration of the nuclear membrane. Such an effect might facilitate contact between a cytoplasmic enzyme and nuclear DNA.

Comparable experiments on the biological effects of enzymes introduced into living cells include the establishment of viral infection in tissue culture cells by viral nucleic acids²; and those where ribonuclease is introduced into plant cells and amoebae¹¹. The phenomenon of metabolic cooperation¹² may involve a similar mechanism.

We suggest that if deoxyribonuclease can enter or be released into the cell nucleus at an appropriate stage of the mitotic cycle, chromosome damage may ensue. Such deoxyribonuclease might be introduced by or from microorganisms, or be an endonuclease redistributed or synthesized within the cell, for instance by effects of radiation or virus infection.

The whole question of the relationship of endonuclease action to DNA replication is of interest. Intact double-stranded DNA is not replicated until a "nick" is introduced by endonuclease, and Kornberg¹³ has reviewed evidence suggesting that replication involves sequential action of an endonuclease, a polymerase and a ligase. The accurate balancing of these enzyme activities is critical for maintaining the integrity of the genome; it therefore seems probable that excess free endonuclease may have deleterious consequences on chromosomal structure, as our results suggest.

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Induction of a Mitochondrial DNA Polymerase in *Tetrahymena*

Tetrahymena pyriformis, strain GL, contains two different DNA polymerase activities—one major and one minor—which can be separated from one another by gel filtration on a 'Sephadex G-200' column¹. Exposure of *Tetrahymena* to various forms of radiation or thymine starvation during growth leads to a rapid increase in the total DNA polymerase activity^{2,3}. The appearance of this new activity is dependent on RNA and protein synthesis^{2,3}. Subsequent experiments have revealed that the rise in total DNA polymerase activity in such cells is the result solely of a marked rise (about 35-fold) in the amount of the minor of the two polymerase activities¹. The activity of the major DNA polymerase remains constant. Here we show that the induced DNA polymerase activity is associated with mitochondria, implying that the induced enzyme is a mitochondrial DNA polymerase.

Cells used for preparation of mitochondria were cultured in a complex medium⁴. DNA polymerase activity was

induced by thymine starvation⁵, as described previously². Mitochondria were prepared from the cells according to published methods^{6,7}, except that the cells were disrupted by low pressure (1,000 lb-inch⁻²) in a French press (American Instrument Co.), and that the various centrifugation steps of the homogenate were repeated several times more than indicated in these procedures. Inspection by electron microscope showed a preparation of essentially pure mitochondria which was only slightly contaminated with cilia. No nuclei could be detected. Cells treated with ethidium bromide were cultured in a defined medium⁸ (enriched with 0.02 per cent proteose peptone) in order to use low concentrations of the drug.

The activities of the two polymerases were measured in optimal conditions¹. The induced polymerase was assayed with heat-denatured DNA primer at an Mg²⁺ concentration of 12 mM, while the nuclear polymerase was measured with native DNA in the presence of 5 mM Mg²⁺. Activity is defined as c.p.m. of ³H-TTP (9.4×10^4 c.p.m./nmole) incorporated into DNA in 20 min. Specific activity is activity per 0.3 mg protein.

Table 1. LOCALIZATION OF THE INDUCED DNA POLYMERASE ACTIVITY IN TREATED CELLS

Enzyme	Activity (per cent)
Total cell extract	100
Sonicated mitochondria	72
Post mitochondrial supernatant	15

The mitochondria were isolated as described in the text. The 100,000g supernatants from the sonicated fractions were used as enzyme preparations.

In vitro DNA synthesis catalysed by the mitochondrial DNA polymerase from rat liver is very strongly inhibited by the dye ethidium bromide, while synthesis catalysed by the corresponding nuclear DNA polymerase is only slightly inhibited⁹. We have investigated the effect of ethidium bromide on DNA polymerase activities from *Tetrahymena*, and found that the induced polymerase activity from thymine starved cells is strongly inhibited by low concentrations of ethidium bromide (4 µg/ml.), whereas the polymerase activity from untreated cells is not. The strong inhibition of the induced activity by ethidium bromide is also observed if native DNA primer

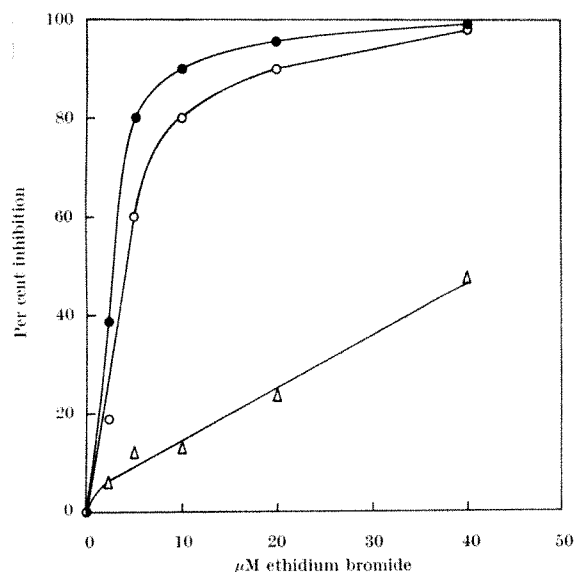


Fig. 1. *In vitro* effect of ethidium bromide on the two DNA polymerase activities. The enzymes were prepared from cells grown in complex medium. One part of the cells was thymine starved; the other part was untreated. The mitochondria were isolated from both cultures and the 100,000g supernatants from the sonicated mitochondria were used as enzyme preparations. The ethidium bromide was incubated for 5 min with the incubation mixture before the enzyme was added. ●, Mitochondrial enzyme from untreated control cells; ○, mitochondrial enzyme from thymine starved cells; Δ, post mitochondrial supernatant from untreated cells.

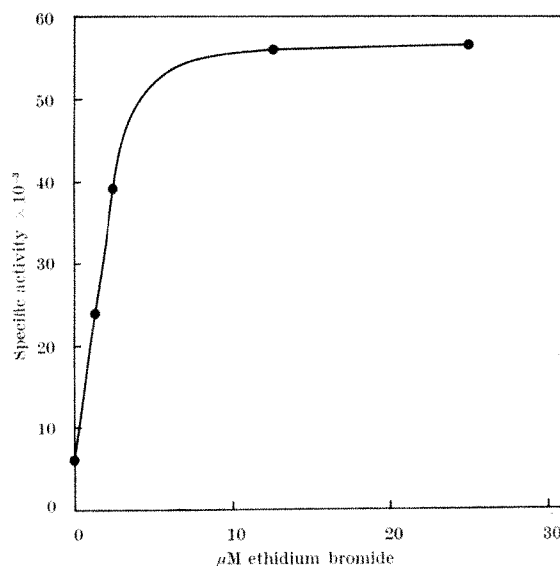


Fig. 2. *In vivo* effect of ethidium bromide on the specific activity of the total DNA polymerase activity. The cells were treated with the indicated concentration of ethidium bromide for 26 h. The 100,000g supernatants were used as enzyme preparations.

is used instead of heat-denatured DNA. Thus the induced polymerase activity is similar to that of mitochondrial rat liver DNA polymerase in response to ethidium bromide. In addition, other properties, such as very high salt optimum for DNA synthesis, are identical for the two enzymes, further indicating that the induced enzyme is probably mitochondrial^{1,10}. We therefore purified mitochondria from thymine starved cells in addition to assaying the various fractions for the induced polymerase activity. Table 1 indicates that the induced activity is purified together with the mitochondrial fraction. In addition isolated nuclei do not contain significant activity (unpublished observations of M. Ikeda and O. W.). Thus we conclude that the induced DNA polymerase in *Tetrahymena* is associated with mitochondria and infer that the induced enzyme is a mitochondrial DNA polymerase. At present we cannot decide whether a new mitochondrial DNA polymerase is synthesized or whether the induced activity is the result of increased synthesis of the DNA polymerase normally present in the mitochondria of *Tetrahymena*. Polymerase isolated from mitochondria of untreated control cells is, however, inhibited by ethidium bromide to the same extent as the induced polymerase isolated from mitochondria of thymine starved cells (Fig. 1).

Both ethidium bromide and acriflavin cause cytoplasmic mutations in certain organisms¹¹⁻¹⁴. At low concentrations of the dyes this mutagenic process seems specific in that only cytoplasmic mutations occur; no nuclear mutations are observed. This indicates that ethidium bromide in such conditions interacts preferably with mitochondrial DNA. Fig. 2 shows that DNA polymerase activity is induced in *Tetrahymena* grown in the presence of low concentrations of ethidium bromide, which might imply that interference with mitochondrial DNA only is sufficient to induce a mitochondrial polymerase. The fact that the induction is completely inhibited by cycloheximide² but not by chloramphenicol (unpublished observations of O. W.) suggests that the induced polymerase is presumably under nuclear control. Thus the system described here might be a potentially useful one for studying nuclear and mitochondrial interaction.

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Adenine-rich Polymer associated with Rabbit Reticulocyte Messenger RNA

POLYPURINES have been isolated from subcellular fractions of mouse liver by a method which involves a limited nuclease digestion of the RNA, followed by chromatography of the digestion products on polystyrene columns¹⁻³. These polypurines consist of clusters of adenylic acid and guanylic acid in which the A/G ratio is 2-10; the materials isolated from the rough endoplasmic reticulum have sedimentation coefficients of 3-5S, but a molecule containing twenty nucleotides can be obtained from the free polysomes⁴. The latter polymer is part of a rapidly labelled RNA species that sediments between the 32S subunit and 5S RNA when EDTA-treated polysomes are centrifuged on a sucrose density gradient. With longer times of labelling with ³²P the polymer is also found in association with the 32S subunit. It seems likely that the adenine-rich polymer is found in close association with messenger-like RNA. These observations prompted us to investigate the polypurine content of a better characterized mammalian messenger RNA. Such an RNA is the haemoglobin messenger RNA which has been isolated from rabbit reticulocytes^{5,6}, and purified by polyacrylamide gel electrophoresis⁷. This species of RNA⁸ and a similar RNA from mouse reticulocytes⁹ have been demonstrated to initiate specific globin synthesis *in vitro*. We now describe the isolation of an adenine-rich polymer from purified messenger RNA obtained from rabbit reticulocyte polysomes and its relationship to the size of the ribosomes from which the messenger RNA is derived.

Ribosomal subunits and the messenger RNA-protein complex¹⁰ were obtained from rabbit reticulocytes essentially as described by Labrie⁷. The different fractions, adjusted to contain 0.1 M Tris-HCl (pH 9.0) and 0.2 per cent sodium dodecyl sulphate (SDS), were extracted at room temperature with an equal volume of distilled phenol previously equilibrated with the same Tris-SDS buffer^{1,3}. The aqueous phase was re-extracted with one-half its volume of the phenol and, after the addition of 0.2 M sodium acetate-acetic acid buffer to adjust the pH to 5, the RNA was precipitated with 2.5 volumes of ethanol at -20° C. After repeated washings with ethanol and finally ether, the RNA was dissolved in 0.03 M Tris-HCl (pH 7.6) containing 0.001 M EDTA and 10 per cent sucrose and subjected to electrophoresis on 2.5 per cent polyacrylamide gels¹¹.

RNA from the 50S subunit was predominantly of the 29S type and included an 18S as well as a 5S peak (Fig. 1a); that from the 30S subunit was predominantly 18S (b). The RNA from the messenger RNA-protein complex showed the presence of a major 10S peak as well as 29S, 18S, 5S and 4S peaks (c). Intermediate RNA species were also present. The specific activity of the 10S messenger RNA was always found to be at least four times higher than that of any other species except 4S RNA.

RNA from the peaks of the different principal species was extracted from the gel slices by homogenizing the gel in 0.1 M NaCl-0.05 M Tris (pH 8.9). The extracted RNA was dialysed against 0.01 M Tris-HCl (pH 7.6) and then incubated with 2 µg ribonuclease S and 16 µg deoxyribonuclease per ml. in 5 × 10⁻³ M MgSO₄ at 37° C for 15 min. After inactivation of the ribonuclease with trypsin, the solution in 0.2 M sodium phosphate (pH 7.6) was applied to polystyrene columns. These retain ribonuclease-resistant polypurines while digested products are eluted by the phosphate buffer^{1,3}. Subsequent elution with phosphate buffer containing 2 mg/ml. of SDS quantita-

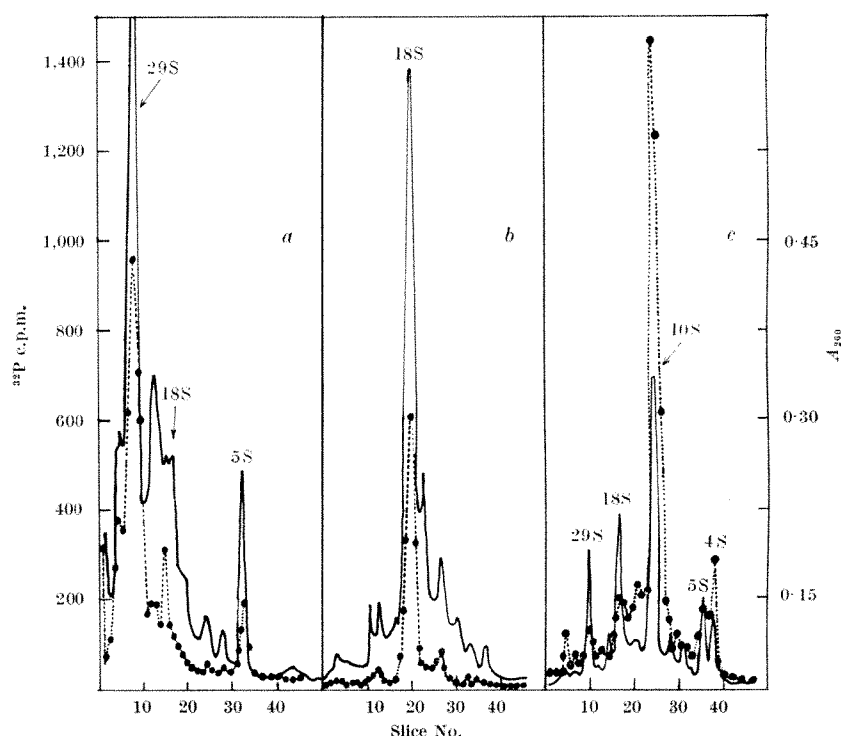


Fig. 1. Electrophoretic analysis of RNA extracted from (a) 50S, (b) 30S subunits and (c) messenger RNA-protein complex of rabbit reticulocyte polysomes. Reticulocytes were obtained from rabbits 18 h after injection of 40 mCi of ³²P-orthophosphate. Electrophoresis was performed at 3.5 mA per tube for 2.5 h at 0-4° C on 2.5 per cent gels. Gels fixed in 4 per cent acetic acid were scanned at 260 nm in a Gilford gel-scanner attached to a Beckman recording spectrophotometer. Gel slices were assayed for radioactivity by measurement of Cerenkov radiation¹² in this and subsequent figures. —, A_{260} ; ●, radioactivity.

tively clutes the polypurines (SDS fraction). This showed that 10 per cent of the counts in the 10S RNA were present as polypurines in the SDS fraction, while the 29S and 18S RNAs had no significant content of these purine-rich polynucleotides. The nucleotide composition of the ribonuclease-resistant component of the 10S RNA was determined by measurements of ^{32}P radioactivity and its size estimated by electrophoresis on 10 per cent polyacrylamide gels (Fig. 2).

The results demonstrated that 10S RNA contained a component with an AMP content of over 70 per cent, contributing 10 per cent of the total radioactivity of the messenger RNA. This adenine-rich cluster was shown to be present in a range of sizes of 50–70 nucleotides on the basis of its mobility compared with that of 4S RNA. An adenine-rich cluster of this size is compatible with the observation by Labrie⁷ that the 10S RNA, containing about 550 nucleotides, had an excess of 75–125 nucleotides over the number required for the coding of either of the two globin chains, and that the extra nucleotides were probably at one end of the messenger RNA molecule. Also consistent with our results is the earlier observation¹⁶ that the nucleotide composition of the messenger RNA determined by ^{32}P analysis showed higher values for AMP than that obtained by spectrophotometric analysis. This discrepancy in values could be explained by a sequence of adenylic acids whose precursor (ATP) had a higher specific activity than the other nucleotides. Another explanation would be that newly synthesized ^{32}P -labelled messenger RNA had a more extensive adenine-rich cluster than the pre-existing messenger RNA and that the adenine-rich cluster was being degraded at a higher rate than the rest of the messenger RNA.

^{32}P -labelled reticulocytes were lysed by the addition of an equal volume of 0.005 M MgCl_2 . Sucrose density gradient centrifugation of the reticulocyte polysomes from the lysate was performed as described by Rich¹⁷. A graded selection of the various aggregates of ribosomes was obtained and RNA extracted from these separate fractions. The RNA of each fraction was then subjected to electrophoresis on 5 per cent polyacrylamide gels which permits selection of the messenger RNA without a previous separation of the subunits. Electrophoretic analysis of the RNA demonstrated clearly the high levels

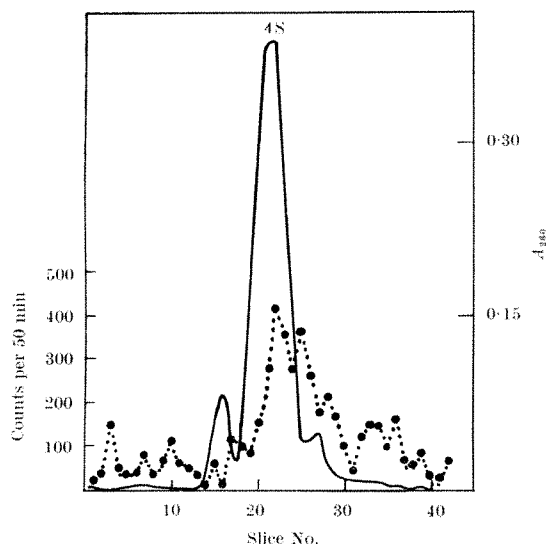


Fig. 2. Characterization of the ribonuclease resistant purine-rich fraction of 10S RNA. Part of this fraction was hydrolysed with 0.3 M LiOH for 18 h at 37°C and after removal of lithium as the perchlorate salt with isopropanol-ether^{3,13} the nucleotides were separated by high voltage electrophoresis¹⁴. The nucleotide composition was: AMP, 70.3; GMP, 11.9; CMP, 3.8; UMP, 14.0. Another part was subjected to electrophoresis in a 10 per cent polyacrylamide gel using 4S RNA as a marker^{14,15} at a current of 5 mA/tube at $0-4^\circ\text{C}$. —, A_{260} ; ● · · · ●, radioactivity.

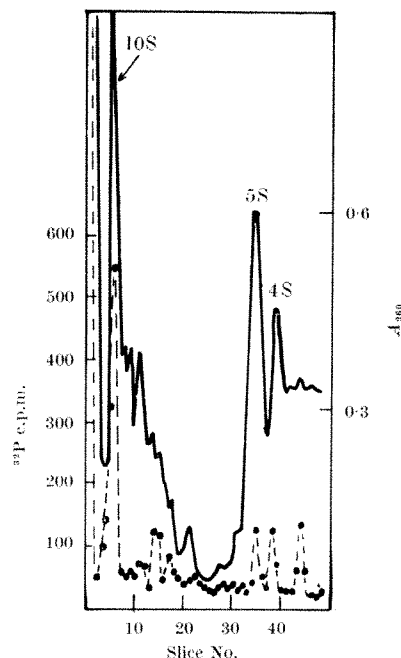


Fig. 3. Separation of 10S RNA of a fraction derived from a sucrose density gradient of ^{32}P -labelled polysomes. Similar analyses were obtained with three other fractions derived from the polysomes on the basis of size. Electrophoresis was performed at $0-4^\circ\text{C}$ on 5 per cent polyacrylamide gels for 2 h, at 4 mA/tube. —, A_{260} ; ● · · · ●, radioactivity.

of radioactivity contributed by the sharp peak corresponding to the 10S RNA compared with that of 4S and 5S RNAs. 18S RNA barely penetrated the gel in the conditions of electrophoresis (Fig. 3). The highly labelled 10S RNA was extracted by homogenization in 0.1 M sodium acetate (pH 7.9) and the RNA precipitated from the high salt medium by cetyl trimethylammonium bromide (CTA)¹⁸. The RNA was converted to the sodium salt and digested with ribonuclease S and deoxyribonuclease as before and the products were separated by polystyrene chromatography to obtain the adenine-rich polymer. After the removal of the SDS by precipitation with KCl, about one A_{260} unit of 4S RNA was added and the RNA was precipitated with CTA. The use of CTA resulted in quantitative recovery of the radioactivity and the RNA samples were then separated on 10 per cent polyacrylamide gels.

Fig. 4 shows that the proportion of the adenine-rich polymer in the various 10S messenger RNAs decreased when the messenger RNA was derived from lighter polysomes or monosomes. This was also reflected as a gradual but definite decrease in the size distribution of the adenine-rich polymer when the messenger RNA was obtained from aggregates of diminishing size, being smallest when obtained from monosomes. It seems as if there is a direct relationship between the size of the adenine-rich polymer and that of the aggregate of ribosomes from which the parent 10S messenger RNA is derived.

The process of maturation of the mammalian reticulocyte is accompanied by a diminishing capacity to synthesize protein and by a progressive loss in ribosomal content, reflected in a decrease in the proportion of larger polysomes in the older cells¹⁹⁻²¹. Accordingly, in a reticulocyte lysate containing the ribosomal contents of a mixed population of maturing reticulocytes, more larger polysomes would be expected to be derived from younger cells and more of the monomers and dimers from older cells, although a considerable overlap would exist. Because the messenger RNA from the larger polysomes has more adenine-rich clusters than the messenger RNA from the smaller polysomes, this variation might be a reflexion of the comparative age of the messenger RNA. The adenine-rich clusters could represent non-translatable residues

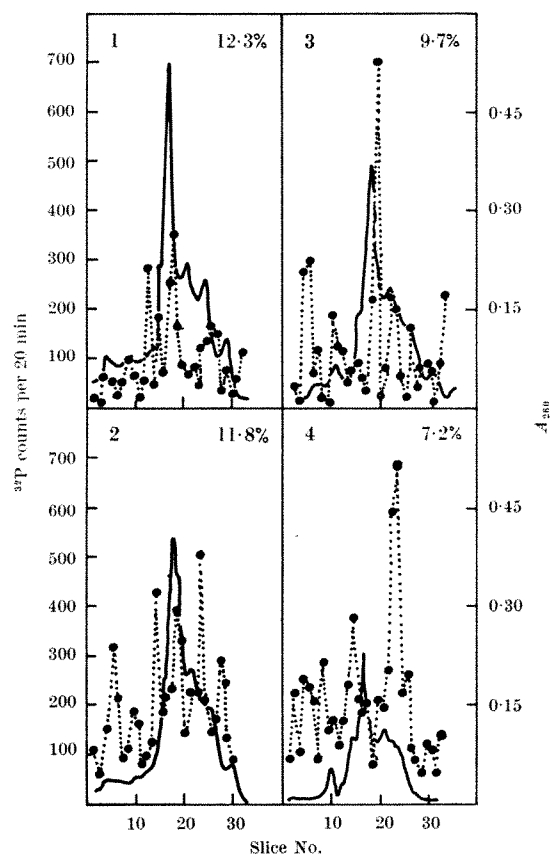


Fig. 4. Electrophoretic analyses of the polypurines of the 10S RNA extracted from the different regions of the polysomes. (1) Polypurines derived from the messenger RNA of the heaviest polysomes; (2) and (3) represent the polypurines of messenger RNA of progressively lighter polysomes; (4) polypurines of the messenger RNA of a fraction consisting mainly of monosomes. The 10 per cent gels were subjected to a current of 5 mA/tube for 90 min at 0–4° C. The proportion of the polypurines in the 10S RNA fraction is shown at top right and expressed as a percentage of the total radioactivity in the 10S RNA fraction from which the polypurines were derived. —, A_{260} ; ●, radioactivity.

that are involved in defining the extent of utilization of the messenger RNA, portions of these adenine-rich clusters being removed successively in each round of the translation process. A similar scheme has been proposed recently by Sussman²² on the basis of entirely different observations. Studies are in progress to determine the fate of these clusters during translation and also to ascertain at which end of the messenger RNA these adenine-rich clusters are located, should they prove to be an integral portion of the messenger RNA.

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Evidence for Immunological Identity of Human and Simian (Baboon) TSH in Serum

IMMUNOLOGICAL cross-reaction as determined by radioimmunoassay has been demonstrated between human and simian growth hormones¹, and between human and simian chorionic gonadotropin². By contrast, there was no evidence of such identity between human and simian luteinizing hormones or follicle stimulating hormones³. Corresponding observations on human and simian TSH have not been reported, but we now present evidence for very close and probably identical immunological cross-reaction between human and baboon TSH on the basis of radioimmunoassay.

Because purified simian TSH is not available, our investigation depended, first, on a comparative study of the changing levels of immunoassayable TSH in human and baboon sera in varying conditions of thyroid hormone excess or deficiency; and, second, on a parallel study of the inhibition curves produced in the immunoassay by the standard preparation of human TSH and by serial dilutions of serum containing high endogenous levels of TSH from hypothyroid human subjects and baboons. The parallelism between these inhibition curves and by serial serum dilutions in these conditions, and with a specific immunoassay, strongly suggests immunological identity.

The radioimmunoassay of human TSH (HTSH) used in these studies is essentially as described previously⁴. HTSH (the gift of the National Pituitary Agency, Endocrinology Study Section, and National Institute of Arthritis and Metabolic Diseases) is labelled with ¹²⁵I (Iso/Serv Division, Cambridge Nuclear Corporation) using the method of Hunter and Greenwood⁵. The antiserum now being used was raised in a rabbit immunized by a single intrasplenic injection of a purified preparation of HTSH in emulsified Freund's adjuvant. It binds 40–50 per cent of a 25 pg label at a final dilution of 1 : 300,000, and cross-

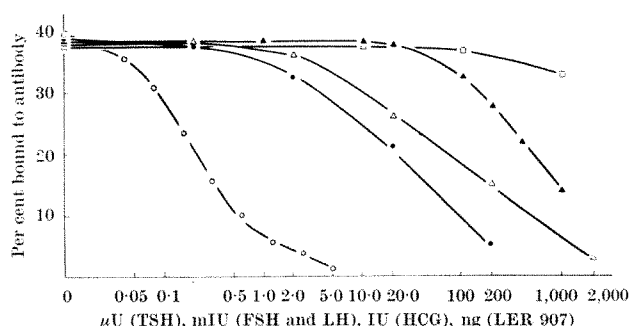


Fig. 1. Inhibition curves in the HTSH radioimmunoassay (¹²⁵I-HTSH, 25 pg; anti HTSH, final dilution 1 : 300,000) produced by HTSH (MRC research standard A) (○), HFHSH (MRC research standard A) (●), HLH (MRC research standard A) (△), LER 907 (human pituitary gonadotropin reference preparation) (▲), and HCG (8,700 IU/mg, Ortho Research Foundation) (□).

reacts with MRC reference preparations of human FSH (HFSH) and human LH (HLH) only at very high and supra-physiological concentrations of these two hormones (Fig. 1). (The observed cross-reaction produced by these reference preparations and by the pituitary gonadotropin reference standard LER 907 may arise in part from contamination with variable amounts of HTSH known to be present in these materials.) Cross-reaction with human chorionic gonadotropin (HCG) does not occur (Fig. 1). This antiserum is now available from the Division of Biological Standards, National Institute for Medical Research, London. All serum levels of TSH are reported with respect to the MRC research standard A HTSH (the gift of Dr Derek Bangham). Separation of antibody-bound and free labelled hormone is carried out using a modification of the dextran-coated charcoal procedure⁶. The assay is sensitive down to 2.0 μ units HTSH/ml. of serum.

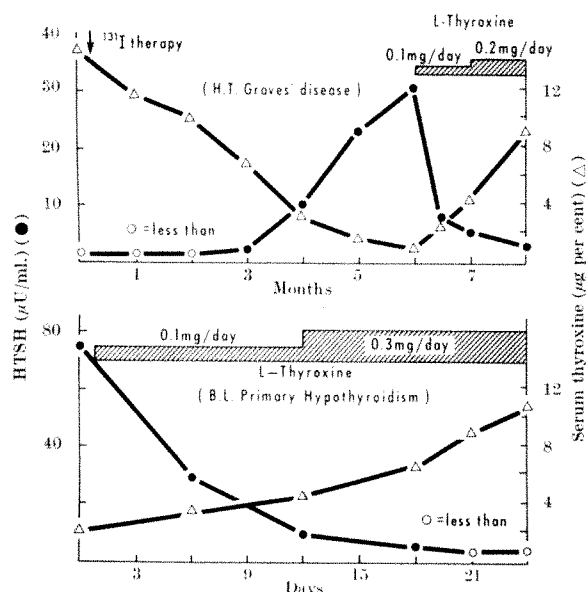


Fig. 2. Serial observations of serum thyroxine and TSH levels in two human subjects in varying conditions of thyroid hormone excess and deficiency.

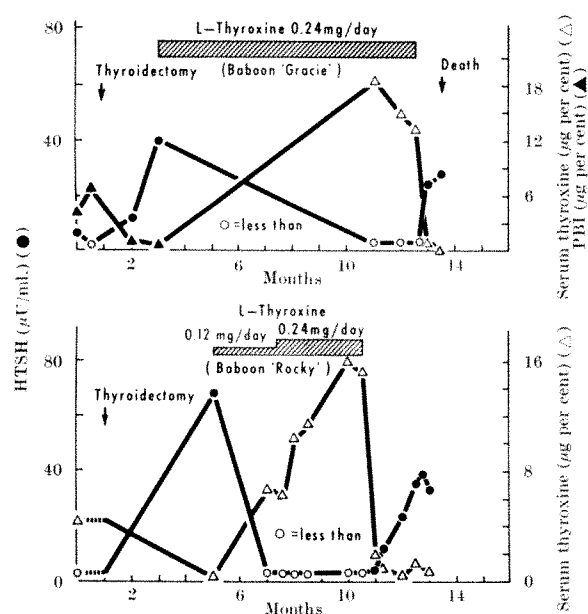


Fig. 3. Serial observations of serum thyroxine and TSH levels in two baboons in varying conditions of thyroid hormone excess and deficiency.

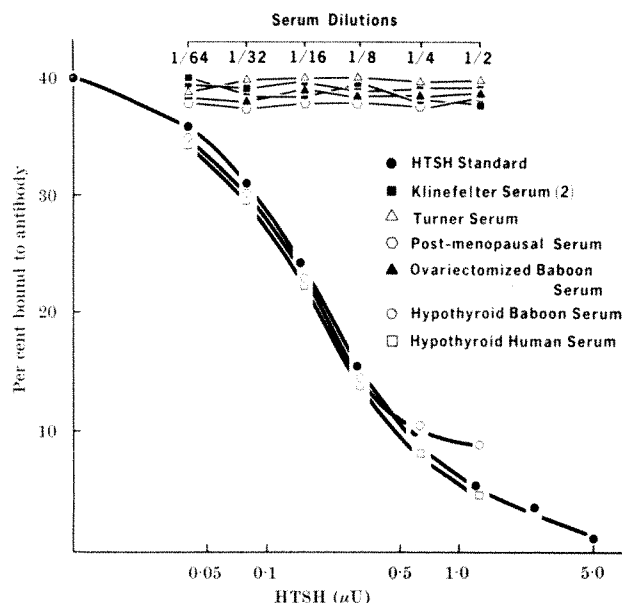


Fig. 4. Inhibition curves in the HTSH radioimmunoassay produced by the HTSH standard and serial dilutions of various human and baboon sera.

Serial measurements of serum levels of TSH and thyroxine iodine (by column chromatography, performed by the Division of Nuclear Medicine, Toronto General Hospital, and by Bio-Science Laboratories, Van Nuys, California) have been made in human subjects and baboons in the following situations: (a) in a patient with hyperthyroidism due to Graves's disease who was treated with radioiodine, subsequently became hypothyroid, and was then restored to a euthyroid state by thyroxine administration; (b) in a patient with primary hypothyroidism who was made euthyroid and then mildly hyperthyroid by thyroid hormone replacement therapy; (c) in two baboons, one male and one female, which became hypothyroid following surgical thyroidectomy, one of these animals subsequently being made hyperthyroid by thyroid hormone replacement, and then again allowed to become hypothyroid following withdrawal of therapy. Additional studies were performed on sera obtained from two adult patients with Klinefelter's syndrome, one adult patient with Turner's syndrome and one postmenopausal woman, none of whom was receiving any form of hormonal replacement therapy. Each of these clinical conditions has been shown to be characterized by high serum levels of pituitary gonadotropins as determined by radioimmunoassays for HFSH and HLH^{7,8}. Serum was also obtained from a baboon ovariectomized two years previously and given no hormonal replacement. It was assumed—no specific assay being available for determination of baboon FSH and LH—that this serum would also contain high levels of pituitary gonadotropins.

Serial determinations of TSH and thyroxine levels in the sera of the two human subjects and the two baboons before and following various forms of therapy are shown in Figs. 2 and 3, respectively. In each case a fall in serum thyroxine is accompanied by a rise in immunoassayable TSH, and vice versa; and a rise in the thyroxine level results in a decrease of serum TSH, often to levels below the minimal sensitivity of this assay. From the baboon studies it seems probable that a considerable time is required (> 2 months) for serum TSH levels to reach a maximum in response to prolonged hypothyroxinaemia. The absolute levels of immunoassayable TSH in the human subjects and in the baboons seem to be comparable in all the conditions of these studies.

Serial dilutions of serum from a hypothyroid human subject and a hypothyroid baboon produced identical inhibition curves in the HTSH immunoassay which were identical

to that for the HTSH standard hormone (Fig. 4). By contrast, no inhibition of the binding of the labelled HTSH to the antiserum was produced either by an equally wide range of serial dilutions of serum from patients with conditions associated with high levels of circulating pituitary gonadotropins, or when serial dilutions of serum were assayed from the ovariectomized baboon (Fig. 4).

We conclude (1) that endogenous TSH in the serum of human subjects and baboons cross-reacts identically in the radioimmunoassay for HTSH; (2) that absolute levels of TSH measured in the serum of humans and baboons by this assay system seem to be similar, and change almost identically with changing concentrations of serum thyroxine; and (3) that FSH and LH present in the serum at presumed high concentration in both humans and baboons do not cross-react in this radioimmunoassay for HTSH.

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Follicle Stimulating Hormone Releasing Activity in Hypophysial Portal Blood and Elevation by Dopamine

FSH-releasing activity, which has been attributed to an FSH-releasing factor (FRF), has been found in hypothalamic tissue¹⁻⁵. It has been assumed that FRF reaches the anterior pituitary through the portal vessels of the hypophysial stalk, but this has not yet been observed in the blood of the portal vessels.

Sawyer *et al.* suggested that neurotransmitter substances are involved in the control of ovulation⁶. Subsequently, many studies have confirmed that the depletion of brain monoamine by reserpine⁷ and the inhibition of catecholamine synthesis^{8,9} prevent ovulation. Moreover, the phenomena of the oestrous cycle, castration, pregnancy or pseudo-pregnancy, which are associated with changes in the levels of sex steroids in the blood, are correlated with changes in the amount of monoamine and the activity of monoamine oxidase in the basal hypothalamus¹⁰⁻¹³. It has been shown that monoamines are present in large amounts in several regions of the mammalian brain and that adrenergic nerve terminals are especially dense in the tuberoinfundibular region of the hypothalamus¹³.

We have observed that there is a marked increase in the LRF activity^{14,15} and prolactin inhibiting factor (PIF) activity (ref. 16 and our unpublished work) in the plasma of the hypophysial stalk after injection of dopamine into the third ventricle of the brain of rats. Intraventricular injections of dopamine into male rats also resulted

in an increase in the plasma concentration of LH^{14,17} and of FSH¹⁸, and a decrease in the concentration of prolactin¹⁶. The infusion of dopamine directly into the anterior pituitary through a cannulated stalk portal vessel had no effect on the release of LH^{14,17}, FSH¹⁸ or prolactin¹⁶. These observations led us to test the hypothesis that dopamine may also stimulate the release of FRF into the blood of the hypophysial portal vessels.

Blood from the pituitary stalk was collected for 2-4 h from male Sprague-Dawley rats weighing 350-450 g¹⁹. For control purposes, blood was obtained simultaneously from the femoral artery of the same animals. The donors of the stalk blood were treated in one of two ways: (1) at the beginning of the collection period, one group of rats was given dopamine hydrochloride (equivalent to 2.5 µg dopamine) in 2.5 µl. of 0.15 M NaCl, the solution being injected into the third ventricle of the brain at a point midway between the optic chiasma and the anterior margin of the median eminence; (2) in another group of animals, the injection was omitted.

Blood plasma from 10 to 12 similarly treated rats was combined, and portions were assayed *in vitro* for FSH-releasing activity. Anterior pituitaries from animals killed by decapitation were bisected along the midline. One pituitary half served as the control, and the contralateral half served as the experimental tissue. All incubations were performed under an atmosphere of 95 per cent O₂ and 5 per cent CO₂ in a metabolic shaker set for 45 rev/min and 25° C. Each pituitary half was placed in a separate flask containing 0.5 ml. of a tissue culture solution ('Difco', medium 199, pH 7.2). After 1 h, the incubation fluid was removed, and blood plasma from the femoral artery was added to one pituitary half. Plasma from the hypophysial stalk was added to the opposite half of the same gland. The incubation was continued for another hour. The quantity of FSH released into the incubation medium by each pituitary half was determined by radioimmunoassay²⁰.

The concentration of FSH in the femoral plasma was undetectable; however, stalk plasma from untreated rats and from rats treated with dopamine contained 0.21 and 0.36 µg/ml., respectively, equivalent to the NIAMD-rat-FSH-RP-1 reference preparation. These quantities were taken into account in the calculation of the results.

During the first hour, when medium 199 was the incubation fluid, the spontaneous release of FSH was 3.85 ±

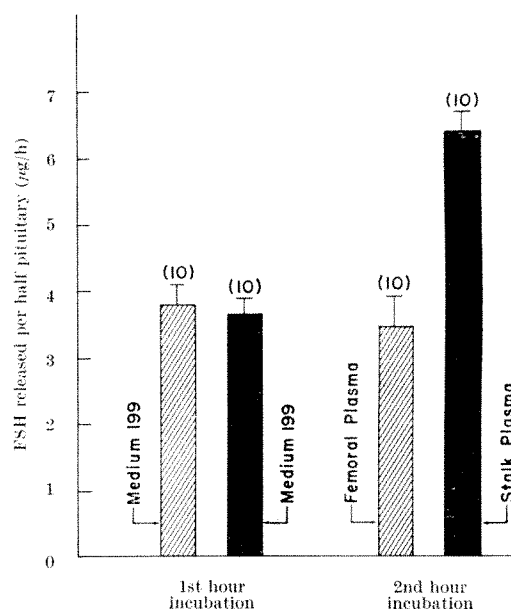


Fig. 1. Effect of hypophysial stalk plasma from untreated rats on FSH release by pituitary halves *in vitro*. The number of halves is shown in parentheses. The vertical lines represent the magnitude of the standard errors.

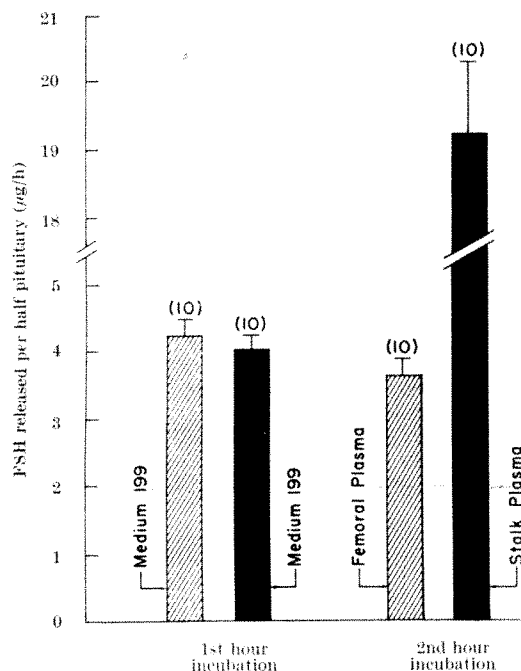


Fig. 2. Effect of hypophyseal stalk plasma from dopamine-treated rats on FSH release by rat pituitary halves *in vitro*. The number of halves is shown in parentheses. The vertical lines represent the magnitude of the standard errors.

0.26 (mean values \pm s.e.; $N=10$) and 3.65 ± 0.26 $\mu\text{g/h}$ by one set of pituitary halves (Fig. 1) and 4.26 ± 0.24 and 4.05 ± 0.21 $\mu\text{g/h}$ by the other set (Fig. 2). When plasma was substituted for medium 199, the rates of FSH release by pituitary halves in femoral plasma were 3.45 ± 0.25 and 3.63 ± 0.26 $\mu\text{g/h}$ (Figs. 1 and 2). These rates were similar to those observed in the first hour when medium 199 was the incubation fluid. The pituitary halves incubated in stalk plasma from untreated animals released 6.34 ± 0.29 $\mu\text{g/h}$ or 1.8 times as much FSH as those incubated in femoral plasma (Fig. 1). The differences between the responses of the paired halves were statistically significant ($P < 0.001$). The halves incubated in stalk plasma from rats treated with dopamine released 19.3 ± 1.20 $\mu\text{g/h}$ or 5.3 times as much FSH as the halves incubated in femoral plasma (Fig. 2). Again, the differences between the responses of the paired samples were significant ($P < 0.001$). The quantity of FSH released by glands incubated in stalk plasma from rats treated with dopamine was also significantly greater than that released by pituitary halves incubated in stalk plasma from untreated animals (Figs. 1 and 2).

These observations show that plasma from the pituitary stalk of the rat contains FSH-releasing activity and this activity in stalk plasma is increased when dopamine is injected into the third ventricle. The FSH-releasing activity may be caused by FRF, the release of which may be regulated by a dopaminergic mechanism.

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4-Threonine-Oxytocin: A More Active and Specific Oxytocic Agent than Oxytocin

SINCE the original synthesis of oxytocin¹, the principal uterine contracting and milk ejecting hormone of the posterior pituitary gland, more than 200 analogues have been synthesized in a number of laboratories and the structure-function relationships of the parent hormone have been elucidated pharmacologically². We now wish to report the synthesis and properties of 4-threonine-oxytocin, an analogue of oxytocin in which the glutamine residue in the four position is replaced by a threonine residue. This analogue has strikingly greater activities in the isolated rat uterus and fowl vasodepressor assays than oxytocin itself, and possesses specifically weaker vasopressor and antidiuretic activities than oxytocin. We believe that study of this analogue may help (a) in elucidating the nature of hormone-receptor interactions, (b) from the standpoint of the phylogeny of the neurohypophyseal hormones, and (c) in clinical use.

The key intermediate required for the synthesis of 4-threonine-oxytocin was the protected nonapeptide, N-benzylloxycarbonyl-S-benzyl-L-cysteinyl-O-benzyl-L-tyrosyl-L-isoleucyl-O-benzyl-L-threonyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-propyl-L-leucyl-glycinamide. This protected nonapeptide was synthesized by the solid phase method³, essentially following the procedure for the synthesis of oxytocin⁴ and 8-phenylalanine-oxytocin⁵. It had a melting point of 240° – 242° C and an optical rotation $[\alpha]_D^{25} -27.5^{\circ}$ ($c=1.0$, dimethylformamide). The amino-acid composition of an acid hydrolysate was Asp₁₋₀₁, Thr₀₋₉₂, Pro₁₋₀₅, Gly₁₋₀₀, Ile₀₋₅₅, Leu₁₋₀₅, Tyr₀₋₇₆, S-benzyl-cysteine, 1.96; ammonia, 2.15. Conversion of this intermediate into 4-threonine-oxytocin was effected as described previously in 4-6; $[\alpha]_D^{25} -10.4^{\circ}$ ($c=0.5$, 1M acetic acid); amino-acid ratios composition: Asp₁₋₁₂, Thr₁₋₀₅, Pro₁₋₀₈, Gly₁₋₀₀, Cys₂₋₁₀, Ole₁₋₀₀, Leu₁₋₁₃, Tyr₁₋₀₅, (NH₃)₂₋₀₄. Thin layer chromatography and paper electrophoresis of the purified material indicated that it was homogeneous. Assay of the 4-threonine-oxytocin by several methods⁷ gave the values indicated in Table 1 and more complete details of the synthesis and pharmacological evaluation will be published elsewhere.

Our pharmacological data clearly differentiate 4-threonine-oxytocin from any reported analogue of oxytocin

Table 1. PHARMACOLOGICAL ACTIVITIES (IN USP UNITS PER mg) OF OXYTOCIN, 4-THREONINE-OXYTOCIN, AND THREE OTHER 4-SUBSTITUTED ANALOGUES OF OXYTOCIN

Oxytocin and analogues	Rat uterus	Fowl vaso-depressor	Rabbit milk ejection	Rat vaso-pressor	Rat anti-diuretic
Oxytocin ²	450	450	450	5	5
4-Threonine-oxytocin	900	1,480	540 (rat = 530)	0.43	3
4-Serine-oxytocin ²	195	230	255	0.1	0.06
4- α -Aminobutyric acid-oxytocin ²	72	108	225	0.1	0.2
4-Valine-oxytocin ⁸	139	230	419	0.005	0.5

solely on the basis of its high oxytocic and fowl vaso-depressor activities and relatively low antidiuretic activity. It is instructive to compare the pharmacological properties of 4-threonine-oxytocin with those of three other 4-substituted oxytocin analogues: 4-serine-oxytocin², 4- α -aminobutyric acid-oxytocin² and 4-valine-oxytocin⁸ (Table 1). 4-Threonine-oxytocin is clearly the most potent and yet this difference must be accounted for by very minor structural differences in the side-chains of the four amino-acids involved: serine, threonine, α -aminobutyric acid and valine. The presence of both the methyl and hydroxyl groups on the β -carbon atom of threonine must be essential for the enhanced activity.

4-Threonine-oxytocin is both more active and more specific than oxytocin in its effects on the rat uterus, and is at least as effective on mammary myoepithelial cells. Thus it has more of the desired physiological properties of an oxytocic hormone and less vasopressin-like activities. If 4-threonine-oxytocin had appeared by mutation during mammalian phylogeny it might be expected to have sufficient adaptive value to survive. It might even occur in some mammalian species, in which case it would not be the first example of a neurohypophysial peptide that was synthesized before its natural existence was recognized: both arginine-vasotocin⁹ and mesotocin¹⁰ were synthesized first and later identified as neurohypophysial hormones.

The course of evolution of neurohypophysial peptides is not clear. Two main lines may have diverged early in vertebrate phylogeny: one contains the 4-serine peptides (glutitocin and isotocin); the other, the 4-glutamine peptides¹¹. A mutation from serine to threonine requires one nucleic-acid base change (UCA/UCG to ACA/ACG). Serine-containing neurohypophysial peptides are only known to occur among cartilaginous and actinopterygian fishes, however, and may not have been present during any stage in the evolution of mammals. A mutation from glutamine to threonine, as would have to occur in the evolution of 4-threonine-oxytocin from oxytocin, requires two base changes, with an intermediate lysine or proline step. Both 4-lysine and 4-proline analogues are very weakly active and would be ineffective oxytocic hormones¹². Thus although 4-threonine-oxytocin might have adaptive advantages as a mammalian oxytocic hormone, it may never have evolved because of the deleterious nature of the necessary mutation.

Clinically the high oxytocic and low antidiuretic activities may give 4-threonine-oxytocin a distinct advantage over oxytocin, assuming that the analogue has similar oxytocic specificity in women. When oxytocin is infused to induce labour, its intrinsic antidiuretic activity causes water retention and, if the infusion is prolonged, may precipitate acute water intoxication. 4-Threonine-oxytocin might be safer.

Deamino oxytocin¹³, an analogue in which the N-terminal amino group is replaced by a hydrogen atom, is also a more potent oxytocic agent than oxytocin. It does not, however, have the same degree of selectivity as 4-threonine-oxytocin, because the antidiuretic potency is also intensified. To our knowledge, this is the first report of the enhancement of activity of a natural peptide hormone by the replacement of a single L-amino-acid.

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Protected Intermediate for the Preparation of Semisynthetic Insulins

PORCINE insulin has only three amino groups, those of the terminal residues of the two chains (glycine^{A1}, phenylalanine^{B1}) and a side chain amino group (lysine^{B29})¹. Many reagents have been developed to take advantage of the reactivity of the NH₂ group in proteins—probably more than for any other functional group—and there have been many reports on the modification of insulin activity by such reagents. Our approach is part of an attempt to develop and use methods which make it unnecessary to synthesize a whole protein when a structure is required which departs only slightly from a naturally occurring one². In the present application to insulin it resembles that of Brandenburg and Ooms³, who used Edman's phenylisothiocyanate (PITC) method⁴ to attack and remove the amino terminal residues of both chains and subsequently coupled an amino-acid back in their place. In this way residues A1 and B1 had both to be replaced with the same amino-acid (glycine). In the course of the procedure, lysine^{B29} was (irreversibly) protected by acetylation.

We report here the use of PITC for specific blocking of one of the α -amino groups as the phenylthiocarbamyl (PTC-) derivative followed by reversible protection of the other two amino groups by the trifluoroacetyl (TFA-) group. The resulting totally amino protected insulin (mono-PTC-, di-TFA-derivatives) can then be treated with anhydrous trifluoroacetic acid (TFA) to remove the PTC-protected residue without affecting the other two protected groups. The value of such a product is that it represents the first requirement in the preparation of many modified insulins. The replacement of amino-acids can involve a different amino-acid, or one substituted isotopically or by a heavy element, or an analogue. Alternatively PITC and TFA can be used to remove the next residue, again without affecting the rest of the molecule, and so on for several

more cycles. Material obtained by more than one cycle of the Edman procedure would offer greater flexibility in resynthesis, for peptides and peptide derivatives could be coupled back, as well as making possible a study on the influence of stepwise removal of residues on biological activity. We have prepared one of the two possible products fulfilling these requirements: [des-Phe^{B1},TFA-Gly^{A1},TFA-Lys^{B29}]-insulin (valine^{B3} with free αNH_2 group) and have made preliminary use of it to bring about a substitution at the amino terminus. The fact that the disulphide bridges have been kept intact throughout places severe constraints on the range of reaction conditions that one can use, but we consider that the freedom from the necessity to re-combine the chains more than justifies the extra effort.

The preparation and use of the derivative take place in a number of stages. First, insulin is reacted with PITC and the required mono-PTC-derivative separated from the other reaction products. The remaining amino groups are then protected and the PTC-residue is removed by cyclization to the phenylthiohydantoin in anhydrous TFA. Replacement of the residue which has been removed is then effected by reaction with an amino-acid activated ester. At all stages it is necessary to be able, if required, to separate derivatives which differ by the number of amino groups acylated and to have a rapid diagnostic method to assess the extent of the reactions. The first need is met by chromatography on DEAE-cellulose in 7 M urea (Fig. 1) and the second by isoelectric focusing in acrylamide gel⁶ (Fig. 2). Finally, solvents must be found in which the desired processes can be carried out without irreversible denaturation of the protein.

The reaction with PITC and the separation of the mono-derivative are described in Fig. 1. Reversible protection of the other two amino groups gives rise to two problems. First, it is essential that the protecting group chosen should be completely stable to strong acid conditions (particularly if the Edman procedure is to be repeated for a number of cycles). Second—a common problem in semi-synthetic work—methods of protection developed for the side chains of free amino-acids are frequently too severe for use in a protein. We considered two possibilities, the phthaloyl group and the trifluoroacetyl group. N-Carboethoxyphthalimide⁷ was found to react with the amino groups of insulin in mild aqueous conditions and was not removed by anhydrous TFA after 45 min at 20° C.

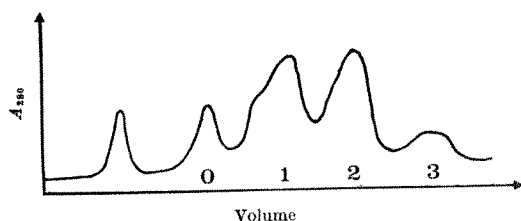


Fig. 1. DEAE-cellulose chromatography at 2° C of the rotary-evaporated products of the Edman reaction in the autotitrator. Insulin (1 g) was dissolved in 100 ml. 1 per cent NaCl at pH 9.5, 40° C, by addition of NaOH. The pH was adjusted to 8.5, 150 μl . of redistilled PITC added and the pH kept constant by the addition of 0.2 M NaOH in an autotitrator. When 175 μl . of the NaOH had been taken up (about 1 h), the reaction was stopped by adjustment to pH 5.3 and the precipitate was washed, collected and dried. This gives rise to a mixture of insulin, mono-PTC-derivative (about 85 per cent on phenylalanine^{B1} and 15 per cent on glycine^{A1}), di-PTC-derivative (almost exclusively on the $\alpha\text{-NH}_2$ groups) and tri-PTC-derivative (peaks 0–3 respectively). Alternatively, particularly if the glycine^{A1} monoderivative is not required, the conditions of Brandenburg⁸ can be used. Here the solvent is pyridine-water (3 : 1) and pH is not controlled. Kinetic studies, monitored by the isoelectric focusing technique, show that the optimum time for production of monoderivative is between 8 and 22 h. 65 g dry weight of exchanger was used in a column of 1.8 cm internal diameter. Flow rate was 40 ml/h. The starting eluent was 0.01 M Tris-0.001 M EDTA-0.03 M NaCl-6.95 M urea (pH 7.7, 20° C). The gradient was linear to 0.1 M NaCl in the same solution. Total eluted volume was 3 l. Better purification of the PTC-phenylalanine^{B1} monoderivative can be achieved by rechromatography of the relevant peak in the starting buffer without a gradient. All such materials are recovered after chromatography by adjustment to pH 5.5, dialysis at 2° C in Visking tubing (18/32 inch) and lyophilization. The identity of the product was established by amino-acid analysis, end group determination (before and after cyclization) and isoelectric focusing.



Fig. 2. Isoelectric focusing by the method of Wrigley⁹, pH 5–7. The faint line (above band 3) is found in all gels containing any protein. a, An artificial mixture of insulin (band 0), mono-TFA-derivative (band 1), di-TFA-derivative (band 2), tri-TFA derivative (band 3); b, purified porcine insulin, showing what are presumably small quantities of des-amido-insulin; c, PTC-Phe^{B1}-insulin; d, product of trifluoroacetylation of c; e, the product in d after cyclization; f, the product in e after coupling; g, the product in f after removal of protecting group.

Unfortunately, in our hands attempted removal of the group by hydrazinolysis in a variety of conditions^{8,9} did not give an adequate yield of deprotected insulin in an undegraded state.

The standard reagent for the application of the trifluoroacetyl group is ethyl thiotrifluoroacetate^{10,11}, but this will not be suitable in the case of insulin because of the attack on the disulphide bridges by the ethane thiol liberated in the protection reaction. Of the alternative trifluoroacetyl esters, phenyl trifluoroacetate¹² seemed most satisfactory. In a typical reaction, 40 mg of purified PTC-phenylalanine^{B1}-insulin was dissolved or suspended in 4 ml. of 0.01 M HCl. 60 ml. of redistilled dimethylformamide (DMF) was added and the water removed by rotary evaporation (0.2 mm Hg, 27° C) of the resulting clear solution to 20 ml. (Insulin and its derivatives frequently will not dissolve directly in anhydrous DMF.) A further 80 ml. of DMF was occasionally added at this point. 1 g of MgO was added to control the reaction and finally 2 ml. of the phenyltrifluoroacetate. The reaction, which appeared to go to completion (Fig. 2), took place for 3 h at 20° C with stirring. After removal of the MgO the protein was recovered by precipitation with ether followed by two ether washes and drying in air.

The complete stability of the TFA-groups to anhydrous TFA was established by exposing tri-TFA-insulin to TFA for 2 h at room temperature. The cyclization of the PTC-group in the TFA-protected derivative was carried out in anhydrous TFA (5 mg protein/ml.) for 1 h at 20° C. The resulting product (obtained by rotary evaporation to 15 mg/ml. and precipitation with ether) is the one sought. It can be separated from any products of partial reaction by DEAE-cellulose chromatography if necessary. If intermediates are to be stored, this is probably the best stage, for the PTC-residue is susceptible to attack by air with a resulting inability to take part in the cyclization process. For the same reason, peroxides must be eliminated from any solvents likely to contain them. The product is successfully de-trifluoroacetylated by MNH_2OH (3 mg/ml.) after 20 h at 20° C. Insulin is known¹³ not to withstand the pH of the more frequently used reagent for the removal of TFA-groups¹⁴, M piperidine (pH 12.4). Even without the rechromatography mentioned, the removal of the protecting groups yields a product forming large rhombohedral crystals of des-Phe^{B1}-insulin by the procedure of Schlichtkrull^{14,15}. There is no need for pre-treatment by extraction¹⁶ or renaturation, but, like the deprotected products of the coupling-reactions, the crystals may take some days to form. The reason for this is being investigated.

Preliminary experiments have been carried out to illustrate the use of the intermediate by reacting it with

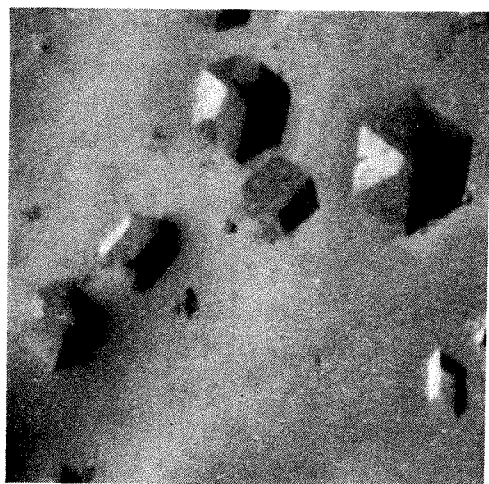


Fig. 3. Crystals of resynthesized insulin ($\times 200$). The *tert*-butoxycarbonyl protected active ester was used in this case. Residues A1 and B29 were deprotected by 1 M NH_4OH , B1 by TFA (2 h at 20°C). The material had the correct end groups and amino-acid composition.

various amino-acid active esters. TFA-phenylalanine hydroxysuccinimide ester has been used, with and without radioactive label on the phenylalanine (^3H , 1,000 mCi/mmol). *Tert*-butoxycarbonyl-phenylalanine hydroxysuccinimide ester has also been used (see Fig. 3). Here, the *tert*-butoxycarbonyl group is not removed with the TFA-group by ammonia treatment. Because, after this treatment, the glycine^{A1} amino group is once more available for reaction while the phenylalanine^{B1} amino group is not, this may provide a more convenient route to modifications of the A chain than the accumulation of the relatively small amounts of PTC-glycine^{A1}-insulin produced in the Edman reaction.

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New Molecular Model for the Long-range Elasticity of Elastin

THE traditional type of elastomer consists of a random network of kinetically free chains linked together by means of stable cross-links: in the case of vulcanized *Hevea*

rubber by sulphur bridges and in the case of the elastic insect protein, resilin, by di- and tri-tyrosine residues¹. The elastic force is caused by the random thermal agitation of the chains whose configurational entropy decreases with strain, while the changes in internal energy remain negligible. Some problems posed by the elastic behaviour of elastin, however, have led us to consider a fundamentally different type of elastomer.

Below 50°C the swelling of elastin in water is very dependent on temperature and the experimental difficulties to which this gives rise were overcome by Hoeve and Flory² using a mixed solvent system (ethylene glycol-water, 30 : 70) in which the swelling of elastin is almost independent of temperature. In these conditions it was found that the isometric force of a stretched piece of elastin is proportional to the absolute temperature, in agreement with the theory for rubberlike molecular networks. It has been pointed out, however, that the use of mixed solvent systems introduces a further complication, for the equilibrium distribution of the solvent components between the elastin sample and the surrounding medium is not independent of temperature³. The swelling of elastin in pure water is almost constant between 50°C and 70°C , and from mechanical measurements performed in this range of temperature it has been concluded that about 85 per cent of the isometric force is caused by changes in configurational entropy while the remaining 15 per cent is the result of changes in internal energy³.

The approach in both investigations^{2,3} is based on an application of the Wiegand and Snyder equation⁴ which presupposes not only that the system is reversible but also that it is free of chemical reactions, as discussed by Weis-Fogh⁵. The experimental results we now report indicate that elastin does not fulfil this condition and that a different approach to the problem seems to be necessary⁶.

Partridge⁷ has suggested a structural model for elastin based on electron microscopic evidence and on results obtained by gel-filtration experiments with columns filled with finely divided elastin fibres. According to this model, elastin is a two-phase system and is composed of globular molecules with the hydrophobic groups packed in the interior and the hydrophilic groups on the surface where they are exposed to the water phase which fills the space between the globules. The globules are interlinked by means of the various cross-links which have been isolated from elastin⁸⁻¹⁰. In elastin the number of amino-acid residues which carry hydrophilic side chains is unusually

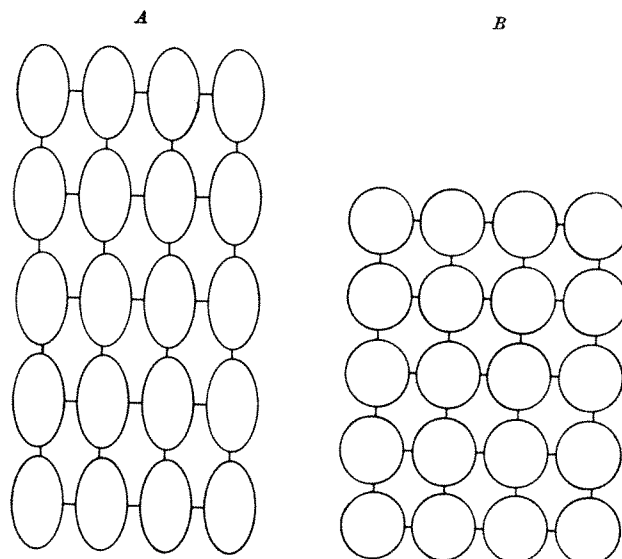


Fig. 1. Structure of elastin as proposed by Partridge⁷. Corpuscular units which have a hydrophobic interior and carry hydrophilic groups on the surface are suspended in a water phase and are interlinked by covalent bonds, A when relaxed, and B when stretched by 35 per cent.

small—only 5 per cent of the total¹¹. If we assume that all the hydrophilic groups are localized at the interface, the area which they occupy is too small completely to cover the surface of the globules, assuming a molecular weight of 67,000 similar to that of tropoelastin¹². In our new functional model, the interface between globules and water should therefore contain both hydrophilic and hydrophobic groups. A diagrammatic picture of the elastin model in the relaxed and in the stretched state is shown in Fig. 1.

When such a two-phase system is strained it is possible that the restoring force is caused by a decrease in the configurational entropy of the chains, as in ordinary elastomers, and by the interfacial tension between the globules and the interglobular solvent, for any deformation of spherical globules will lead to an increased area of the interface. Such an increase in area must mean that hydrophobic groups are brought from the interior of the spheres to the surface layer where they become exposed to the surrounding water. Because transfer of hydrophobic groups from a hydrophobic environment to water is known to involve a considerable decrease in entropy of the system, in the globular structure discussed here deformations can be expected to cause a decrease in entropy larger than that found in elastomers, and which is based solely on changes in configurational entropy.

We have determined the heat exchange which occurs when small samples of purified elastin were stretched and relaxed. To decide whether elastin behaves according to the theory for rubbers or whether hydrophobic interactions could be involved, the mechanical work was compared with the heat exchange. The elastin was prepared from bovine ligamentum nuchae in the form of thin strips which were purified by repeated autoclaving according to Partridge *et al.*¹³. The microcalorimeter used was originally designed by Clarke and Rothschild¹⁴. The reaction chamber has a capacity of 2.3 ml. and an elastin sample could be suspended inside it in such a way that it could be stretched and relaxed from the outside by means of a thin silver chain. The calorimeter is suitable for measuring rates of heat production, dQ/dt , of the order of 1 mcalorie/h. When the heat production is about 3–5 mcalories and when it takes place in less than a minute after the deformation, the calorimeter can be used as an integrating device where the total amount of heat produced or absorbed,

$$Q = \int_{t=0}^t dQ/dt$$

can be estimated within about 5 per cent in the course of about 15 min. The optimal sample size is a strip 15–20 mm long and about 3 mm² in cross-section, correspond-

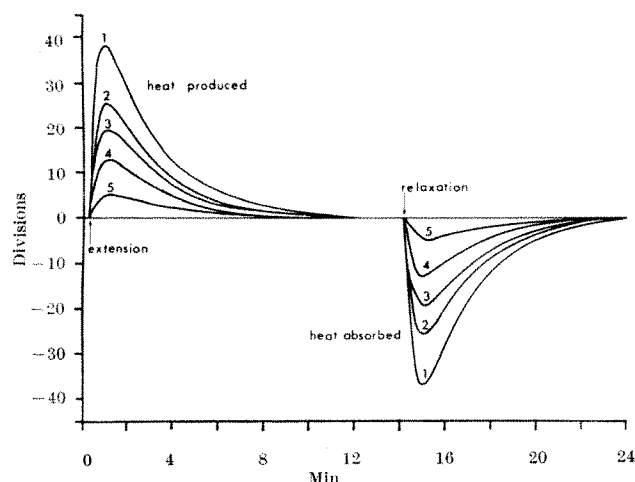


Fig. 2. Heat exchanges measured in the calorimeter when a sample of elastin is extended (stretched) or relaxed at room temperature in various solvents. (1) in water, (2) in 2 M methanol, (3) in 2 M ethanol, (4) in 2 M *n*-propanol, (5) in pure formamide. One division on the ordinate equals 0.046 mcalorie/min.

Table 1. HEAT EXCHANGE Q AND MECHANICAL WORK WHEN ELASTIN SAMPLES WERE EXTENDED AND RELAXED IN BUFFERED WATER AT ROOM TEMPERATURE

Sample	Mechanical work (mcalories)	$Q(\text{extend})$ (mcalories)	$Q(\text{relax})$ (mcalories)	Ratio $Q(\text{relax})/Q(\text{extend})$
1	1.33	-5.53	+5.60	1.01
2	1.02	-5.60	+6.10	1.09
		-5.71	+5.32	0.93
		-6.13	+6.00	0.98
3	1.08	-5.73	+5.50	0.96
		-5.92	+5.41	0.91

A positive sign means that heat was absorbed by the system.

ing to about 35 mg dry weight. Fig. 2 shows a typical heat response obtained on stretching and relaxing such a sample.

In a series of measurements (Table 1) it was found that the heat produced during stretching always exceeded that corresponding to the mechanical work involved. It was also almost equal to the heat absorbed when the sample was relaxed again, so that the processes are fully reversible. Because the stress-strain curves were also reversible the sample plus the surrounding solvent in the reaction chamber can be considered as a closed, reversible thermodynamic system working at constant temperature and pressure and with very small volume changes. The reversible uptake of heat, $Q^{(\text{rev})}$, is equal to $T\Delta S$, and the work done by the system on the surroundings, $W^{(\text{rev})}$, is equal to $-\Delta F$ which is the change in free energy. The fundamental equation for a closed system at constant temperature is $\Delta F = \Delta U - T\Delta S$, so that we can estimate the change in internal energy, ΔU , by inserting the two measured quantities: $\Delta U = Q^{(\text{rev})} - W^{(\text{rev})}$.

From the results in Table 1, ΔU values of between -4 and -5 mcalories are found, whereas the mechanical work corresponded to an increase in free energy of only 1.0 to 1.3 mcalories for samples stretched by 35 per cent. The results therefore indicate that deformation of elastin in water involves reversible chemical changes which are several times larger in terms of energy than the corresponding mechanical changes. In this respect elastin differs fundamentally from an ordinary rubber.

In an attempt to characterize the process further we changed the medium surrounding the sample to see if this would alter the heat exchange. It was found that changes in pH have very little effect: within the pH range 2–10 the highest value measured was 6.81 mcalories at pH 10 and the lowest value was 6.08 mcalories at pH 4, indicating that charged groups are not involved to any significant extent. Fig. 2 shows that the heat exchange is strongly influenced by the presence of organic solvents in the medium and that the effect is larger with alcohols having longer hydrocarbon chains than with those having shorter hydrocarbon chains. The heat exchange is also fully reversible in these circumstances, and it returns to its original high value when the sample is transferred back into water. The concentration of alcohol in the medium is important, as shown in Fig. 3. At ethanol concentrations exceeding 30 per cent, the entropy term changes sign so that there is a heat uptake instead of a heat output during stretching. In ethylene glycol-water (30 : 70)—the medium used by Hoeve and Flory²—the heat output was nearly equivalent to the work performed during stretching, indicating that the change in internal energy in this system is close to zero.

The effect of temperature is illustrated in Fig. 3, which shows that in water the heat of stretching decreases with increasing temperature. Again, these changes are fully reversible. Fig. 4 illustrates the pronounced shrinking of water-swollen elastin at elevated temperatures and it also shows that the stress-strain curves remain almost unchanged, indicating that the amount of work performed during a given increase in length does not alter much with temperature from 2–87°C. The change in internal energy must therefore decrease drastically with temperature because of the entropy effect. At 60°C, ΔU is about

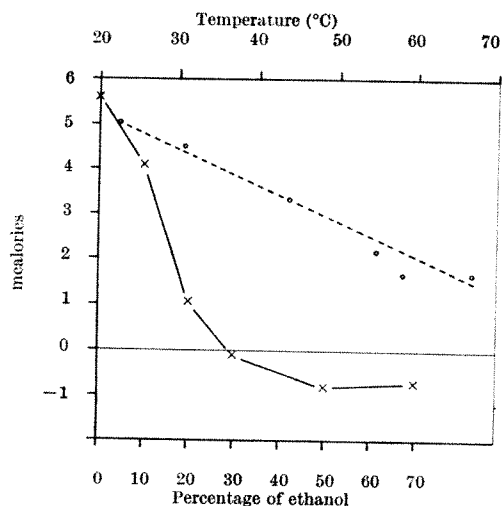


Fig. 3. The effect of the concentration of ethanol on the heat produced when elastin is stretched at room temperature (\times — \times). The effect of temperature on the heat produced when elastin is stretched in water at pH 7 (phosphate buffer) (\circ — \circ).

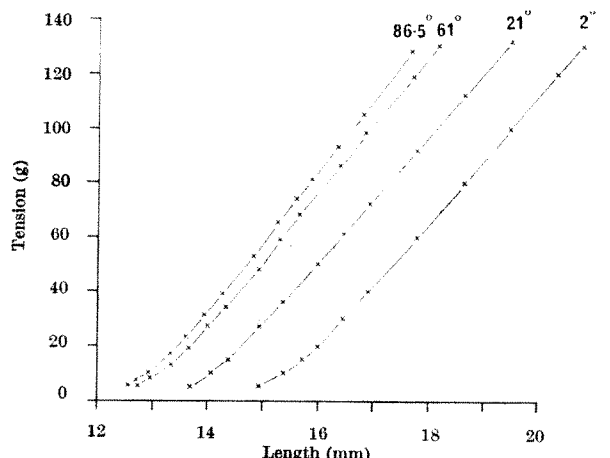


Fig. 4. Length-tension curves for a sample of elastin stretched in water at different temperatures ($^{\circ}$ C).

1 mcalorie, or of the same magnitude as the mechanical work.

We conclude that the theory for rubberlike elasticity is inadequate to explain the thermodynamic behaviour of elastin, whereas it remains to be established whether the observations are compatible with the globular model—involving interfacial forces. By assuming spherical globules of a given size which are stretched into prolate spheroids of the same volume, it is possible to calculate the increase in surface area and to estimate the interfacial tension which is necessary to account for the work measured. One can also estimate the number of hydrocarbon groups which can be accommodated in the extra interface generated by the stretch and calculate the change in entropy involved in the transfer of the groups from a non-polar phase to a polar environment. Such calculations can only give the order of magnitude of the changes to be expected, because of the simplifying assumptions which are necessary, but they will show whether the corpuscular model and interfacial forces should be taken seriously or not.

On transforming a sphere into a prolate spheroid of unchanged volume, the relative increase in surface area is given by the expression $A/A_0 = \frac{1}{2}(\lambda^{-1} + \lambda^{1/2} \sin^{-1} \sqrt{1 - \lambda^{-3}} / \sqrt{1 - \lambda^{-3}})$, where A_0 is the unstrained area and λ the extension ratio (see Fig. 5). The area changes slowly at first and later it increases almost linearly with λ . If a sphere is stretched from $\lambda = 1.00$ to $\lambda = 1.35$, its surface area increases by 3 per cent. Dense protein globules of a

molecular weight of 67,000 have a diameter of about 54 Å and a surface area of about 9000 Å², so that the corresponding increase in surface area of a globule is 270 Å². The total increase in area in samples weighing 35 mg is 8,400 cm². If we assume that the total work performed during stretching (1 mcalorie) is caused entirely by this increase in surface, the necessary interfacial tension amounts to 5 dyne/cm, which seems to be reasonably small: the interfacial tension between water and diethyl ether is 10.7 dyne/cm and between water and *n*-octyl alcohol is 8.5 dyne/cm at 20 $^{\circ}$ C (ref. 15). The increase in area corresponds to the transfer of maximally 1.1×10^{-5} mole CH₃ groups from the core of the globules to the surface, each CH₃ group having a diameter of 3.7 Å. The transfer of a methyl group of an alanine side chain from a non-polar phase into water at 25 $^{\circ}$ C is accompanied by the following changes: $\Delta F_{tr} = 1.3$ kcalorie/mole and $\Delta S_{tr} = -9.4$ entropy units/mole¹⁶. In the case of the reversible transport from bulk solution into the surface we shall assume that the side chains do not become completely surrounded with water but that the actual figure amounts to half the value measured for the transfer from bulk phase to bulk phase. For the elastin samples used in this investigation we should therefore expect: $\Delta F_{tr} = 7.2$ mcalories, $T\Delta S_{tr} = -15.4$ mcalories, and $\Delta U = -8.2$ mcalories.

In other words, surface interactions between hydrophobic and hydrophilic phases can give rise to changes in entropy, free energy, and internal energy which are of the same sign and order of magnitude as those measured experimentally.

According to published figures¹⁶, an increase in temperature should result in a larger ΔF_{tr} and a numerically smaller $T\Delta S_{tr}$, so that ΔU is reduced numerically. Then interfacial interactions should contribute to the isometric force by larger amounts at the high than at the low temperatures used, whereas the heat exchange with the surroundings should decrease. This is essentially what we have found (Figs. 3 and 4).

The measurements and calculations are therefore in agreement with the hypothesis that water-swollen elastin is a two-phase system and that part of the elastic force is caused by interaction between the hydrophobic groups in the protein and the water at the interface. Part of the elastic force must, however, arise from thermal agitation of the protein chains in the interior of the globules, because in all conditions the chains must be able to move freely relative to each other and become orientated during stretching. Although we cannot yet estimate the contribution to the total stress caused by changes in configurational entropy and by surface interaction, it is worth noting that the actual force-extension curves for elastin swollen in water (Fig. 4) resemble the area-exten-

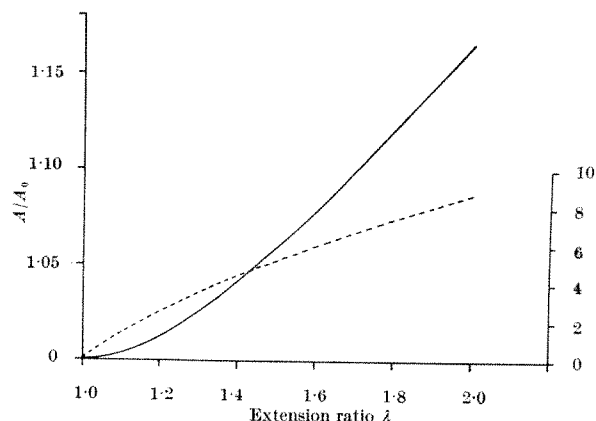


Fig. 5. The relative surface area, A/A_0 , of a sphere of constant volume which is deformed into a prolate spheroid by a unidirectional force (—) (see text). Tensile force, f , as a function of λ , as calculated for a swollen isotropic rubber according to the formula $f = Gv^{1/2}(\lambda - \lambda^{-2})$, where G is the modulus (here 6.4 kg/cm) and v the volume ratio (here 0.45) (---).

sion relationship in Fig. 5 more than they resemble the typical stress-strain curve for an ordinary rubber (broken curve in Fig. 5).

It does not seem to have been suggested before that elastomers can operate on the basis of interfacial forces, and we propose to call such an elastomer a liquid drop elastomer. In the ideal case, the interior of the drops would be completely fluid so that no orientation could be imposed on it during deformation of the droplets; the interfacial forces would be the only restoring force. The ideal case will be difficult to obtain but it should be possible to prepare synthetic elastomers of the mixed type in which both interfacial forces and changes in configurational entropy contribute to the elastic force, as suggested here for elastin.

In biology, one advantage of a liquid drop elastomer is that one can imagine the existence of elastic fibrils of much smaller diameter than is possible for rubberlike elastomers, because a single linear row of interlinked globular molecules would show an elastic behaviour which is similar to that of a larger aggregate of globules. Such elastic microfibrils could contribute to the mechanical behaviour of many cell types although their presence would be difficult to demonstrate.

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Role of Macrophages in the Development of Immunological Maturity in Rat

MOST neonatal mammals are unable to produce antibodies after antigenic stimulation possibly because of a deficiency in a link in the chain of events which leads to the production of antibodies. The aim of this study was to ascertain the part played in the immunological immaturity of infant rats by the inability or deficiency in antibody production by macrophages.

Peritoneal cells were collected by rinsing the peritoneal cavities of adult rats without previous treatment with heparinized Parkers medium. The washings were kept ice cold and centrifuged for 5 min at 1,500 r.p.m. The cells of the sediment were counted, their viability was checked by the trypan blue exclusion test and they

were then stained for differential counting. Appropriate amounts of cells (from 12 to 36×10^6), suspended in 0.05 ml. Parkers medium with 20 per cent horse serum, were injected intraperitoneally into one day old Wistar rats; several rats of each litter, injected with the suspending medium without cells, served as controls. Two days later, all the rats (experimental and controls) were injected intraperitoneally with 0.05 ml. 20 per cent sheep red blood cells (SRBC). The peritoneal cells used for inoculation consisted of 70 per cent macrophages (60–82 per cent), 6 per cent lymphocytes (3–9 per cent) and 21 per cent granulocytes (12–30 per cent). Their viability was more than 90 per cent. Blood was drawn by heart puncture every 3–4 days, beginning from the fourth day of immunization, and the haemolysin content of the serum was determined by the micromethod of Takatsy. In several experiments, the infant rats were killed on the fourth day of immunization and their spleens were examined for plaque forming cells (PFC) after the method of Jerne modified by Sterzl. The results are shown in Table 1. The use of rats for this experiment is convenient because they do not have natural antibodies to SRBC, therefore no passive transfer of maternal antibodies can occur. Our rats can produce antibodies when twelve days old.

Table 1. ANTIBODY RESPONSE OF INFANT RATS TO SRBC AFTER INOCULATION WITH MACROPHAGES FROM ADULT RATS

Series	No. of infant rats	No. of macrophages injected $\times 10^6$	No. of rats showing haemolysins
1	6	12	6
	4	0	0
2	5	12	5
	2	0	0
3	5	13	2
	4	0	0
4	3	14	2
	3	0	0
5	5	16	2
	3	0	0
6	3	26	3
	2	0	0
7	3	36	3
	2	0	0
8	2	13*	2
	2	0	0
9	3	13*	3
	2	0	0
10	2	13*	2
	2	0	0

* Macrophages detached after incubation on glass dishes.

Thirty of thirty-seven inoculated infant rats, which were injected with adult peritoneal cells before the antigenic stimulus, produced haemolysins in a titre of 1:4–1:512. The titre began to rise on the fourth day after the antigen and reached the peak on day 10–15. PFC (3–63/spleen) were found in the spleens of eight rats that were killed on the fourth day after the antigen. The controls which had been inoculated simultaneously with SRBC but without macrophages did not respond to the antigenic stimulus, neither by elaborating haemolysins nor PFC.

In several instances, the peritoneal cells were incubated on glass dishes, detached after 1 h with a rubber policeman and rinsed before being used to inoculate infant rats; the detached cells, devoid of non-adherent elements (that is lymphocytes and granulocytes), also induced an antibody response in infant rats.

It seems justified to assume therefore that adult, functionally adequate macrophages are the necessary prerequisite for initiating the production of antibodies to SRBC in infant rats.

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Biochemical Changes during Learning in an Insect Ganglion

COCKROACHES (*Periplaneta americana*) can be mounted in such a way that when one animal places its metathoracic leg in a solution, it gets a shock at the rate of one per second. A second, control, animal gets a shock each time that the experimental animal is shocked regardless of the position of the metathoracic leg of the control animal. The experimental animal learns within 35 min to keep its leg out of the solution whereas the control animal does not learn to raise its leg, even though both animals received the same number of shocks¹⁻⁴.

We have used this system to study some of the chemical changes that take place in the metathoracic ganglion during the learning process and have compared the ganglia of experimental animals, control animals and resting animals (the latter receive no shocks). Injection of drugs such as cycloheximide⁵, acridine orange, congo red or actinomycin D slows up the learning process; the experimental animal requires more shocks and a longer time (up to 60 min) to satisfy the learning criteria. A dose-response relationship can be established between the amount of drug injected and the time that the animal requires to learn. Conversely, injection of edrophonium, prostigmine or magnesium pemolate reduced the number of shocks and time required for the animal to learn (15 min) and a corresponding dose-response relationship can be established.

The chemical changes taking place in the ganglia were studied as follows. Experimental animals were injected with ¹⁴C-uridine and the control animals with ³H-uridine immediately before the training process. Both groups of animals were then trained. After training, the metathoracic ganglia were removed, pooled and homogenized and the RNA was extracted. The carbon/tritium ratio in the RNA was determined and showed that the experimental animals had a greater incorporation of labelled uridine into their ganglionic RNA than did the control or resting animals:

Experimental > control by 44.00 ± 5.10 per cent ($n = 20$)
Control > resting by 32.25 ± 4.38 per cent ($n = 11$)
Experimental > resting by 68.80 ± 15.89 per cent ($n = 7$)

Autoradiographic studies on metathoracic ganglia showed that the experimental ganglia incorporated more radioactivity into the nerve cells at the posterior region of the ganglion, this being the region associated with leg movements⁶.

We have developed a quantitative method of estimating the distribution of labelled uridine in the experimental and control ganglia. ³H-Uridine was injected and animals were trained as described. The metathoracic ganglia were removed, dehydrated and waxed and normal histological blocks were prepared and sectioned at 20 μ m, transverse to the ganglion. Each section was placed in a vial containing scintillation fluid. The fluid dissolved away the wax and the activity in the vial was then counted. Fig. 1 illustrates a comparison of the activity in sections of the experimental ganglia and the control ganglion. The experimental ganglion incorporated more material and there was more activity in the posterior region of the ganglion, in agreement with the autoradiographic picture. Sucrose density gradient analysis of the labelled RNA in the ganglia showed that the experimental ganglia incorporated more labelled uridine into the lower molecular weight RNA fractions. The experimental ganglia also incorporated more labelled leucine than did the control or resting ganglia.

The action of drugs on the learning rate gives some indication of a possible site of action. Because anticholinesterase agents such as prostigmine or edrophonium made the animals learn more quickly, we measured the cholinesterase level in the experimental, control and resting metathoracic ganglia: the results are shown in Table 1.

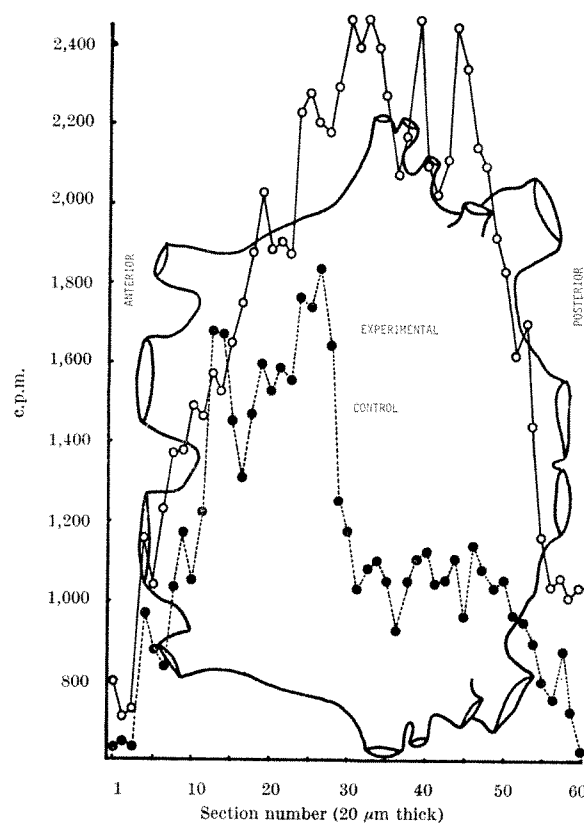


Fig. 1. Location of radioactivity in experimental control ganglia. The ganglion was embedded in wax and cut into sections 20 μ m thick. The activity in each section was counted. Experimental ganglia had the higher activity, more activity being present in the posterior end of the ganglion (to the right of the figure).

The metathoracic ganglia of the experimental animals had a much lower cholinesterase activity than the ganglia of the control or resting animals, the value being approximately one-third of that for the resting animal. This change in cholinesterase activity had taken place during the 30 min of the training process. A study of the rate at which the cholinesterase activity returned in the ganglia of the experimental animals showed that it took 3 days to return to the resting value. Simultaneous tests of the number of shocks required to bring another set of experimental animals back to the learning criteria during the three days after training showed that after three days the animal had effectively forgotten. Fig. 2 illustrates the recovery of the cholinesterase activity and the loss of memory of the animals. We have evidence indicating that the experimental ganglia produce less γ -aminobutyric acid (GABA) from glutamate than do the control or resting ganglia. Acetylcholine is probably the excitatory transmitter at the cockroach central nervous synapse and GABA is probably the inhibitory transmitter⁷.

Our results indicate that, among other changes, cholinesterase activity decreases markedly in the experimental ganglia on learning. This would facilitate synaptic transmission and could alter the synaptic ratio from an input: output ratio initially of, say, 20:1 to a value of 2:1 or even 1:1. The decrease in the level of GABA might also increase the likelihood of transmission, so that specific pathways would be facilitated. We interpret the difference between the experimental and control ganglia to mean that although both animals receive the same number of electrical shocks, there are some neurones in the experimental animals that receive a patterned sensory

Table 1. CHOLINESTERASE LEVELS

Metathoracic ganglion	μ moles acetic acid released/h/100 μ g protein
Experimental	1.22 ± 0.08 ($n = 21$)
Control	2.43 ± 0.19 ($n = 21$)
Resting	3.42 ± 0.53 ($n = 21$)

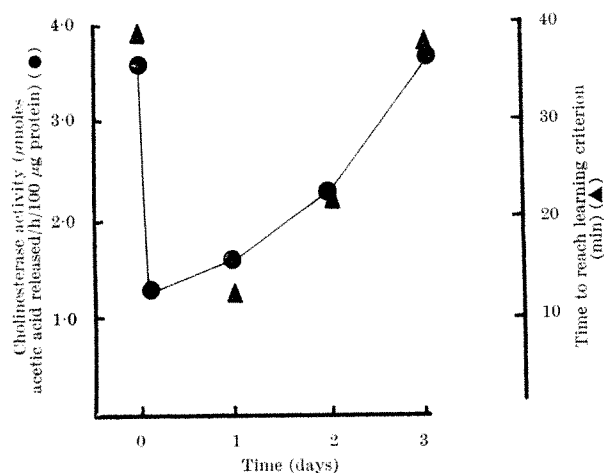


Fig. 2. Change in cholinesterase activity in the ganglion and the retention of learning. On training at day 0 the cholinesterase level in the experimental ganglion diminished rapidly, slowly returning to the normal level after 3 days (●—●). The experimental animals slowly forgot their training (▲—▲) during the 3 days and required an increasing number of shocks to reach learning criterion.

input simultaneously with the shocks; these neurones will receive greater stimulation and so effect a greater change in the level of cholinesterase and production of GABA involving specific pathways. Our demonstration of a greater turnover of RNA, enhanced protein synthesis, a rapid decrease in cholinesterase activity and a slower production of GABA in experimental animals leads us to suggest that the latter two changes constitute the basis of short term memory storage (1–3 days). Moreover, the changes in protein synthesis may bring about morphological changes that would be responsible for longer term memory.

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Destruction of Lymphoid Cells by Activated Human Lymphocytes

THE mitotic activation of lymphocytes *in vitro* by histocompatibility antigens has some surprising features. Human lymphocytes are activated by isoantigens on fresh or X-irradiated lymphocytes of another donor (the mixed lymphocyte reaction), but not by isoantigens on human fibroblasts and HeLa cells, or on leucocytes killed in various ways, or on leucocyte fragments^{1–3}. The mixed lymphocyte reaction has been shown, in rats, to be immunological in nature. It is specifically abolished when tolerance is produced by neonatal injection of bone marrow cells bearing the isoantigens^{4,5} and is a primary response; yet approximately 2 per cent of the cells in the circulating lymphocyte population, in the rat, are directly responsive to lymphocytes bearing isoantigens associated with any one of a series of alleles present at the major histocompatibility locus⁵. Human lymphocytes are activated by X-irradiated cells from various human

Table 1. CYTOTOXIC ACTIVATION AND DNA SYNTHESIS OF CULTURED LYMPHOCYTES OF AN ADULT

Stimulant added to culture	Days of culture	Percentage ⁵¹ Cr release		³ H-thymidine incorporation (d.p.m.)	
		Test*	Control†	Test	Control
None	7	9.2	10.4	883	224
X-Irrad. EB2 (2 × 10 ⁵)	7	32.1	8.4	63,773	1,911
X-Irrad. EB2 (10 ⁵)	7	20.5	10.0	70,736	672
X-Irrad. EB2 (2 × 10 ⁴)	7	11.9	10.5	1,857	336
Staphylococcal filtrate (0.1 ml.)	7	10.9	9.3	3,834	355
None	8	15.3	14.3	826	750
X-Irrad. EB2 (2 × 10 ⁴)	8	51.2	14.8	33,975	2,567
X-Irrad. EB2 (10 ⁵)	8	26.7	11.8	37,200	752
X-Irrad. EB2 (2 × 10 ⁴)	8	15.1	14.1	1,006	487
Staphylococcal filtrate (0.1 ml.)	8	14.3	15.2	2,059	570

* Test = culture (1 ml.) containing 2 × 10⁶ blood lymphocytes from an adult human donor in 20 per cent pooled human serum-gelatin in Eagle's MEM.

† Control = culture (1 ml.) matched to contain the same number of red cells and the same stimulant but lacking blood lymphocytes.

EB2 cells were labelled by incubation with Na⁵¹CrO₄ (1 μCi per 10⁶ cells) for 24 h and washed. The percentage release of ⁵¹Cr was determined after a 6 h period of incubation at 37° C with ⁵¹Cr-labelled EB2 cells. Maximum release on freezing and thawing = 80 per cent. ³H-Thymidine incorporation was determined after 24 h incubation with 0.5 μCi of ³H-thymidine as previously described¹; d.p.m. = disintegrations per minute. EB2 is a Burkitt lymphoma cell line. X-irrad. = X-irradiated with 6,000 r. All results are means of triplicates.

lymphoid cell lines (LCL) in a reaction closely similar to a unidirectional mixed lymphocyte reaction, but of considerably greater intensity^{1,2,6,7}. Cord blood lymphocytes, as well as lymphocytes of the adult, are responsive and a high proportion of the blood lymphocyte population is sensitive to antigens on the LCL cells. These experiments demonstrate the inbuilt reactivity of lymphocytes to certain antigens and the importance of "presenting" the antigens in the right way to the lymphocytes. Some cells seem to "present" their surface antigens in a way which "triggers" lymphocytes whereas others do not, a fact of practical importance in attempts to demonstrate tumour antigens by mixed cell tests.

The thymic-derived lymphocytes activated in the mixed lymphocyte reaction are thought to be effector cells with cytotoxic properties⁸. In human or pig mixed lymphocyte reactions, however, there is little indication of inter-destruction in survival tests based on dye-exclusion. Human lymphocytes stimulated with allogeneic lymphocytes in culture have been shown to be cytotoxic to Chang cells (human liver)⁹, but human lymphocytes were not damaged when exposed to a hundred-fold excess of allogeneic lymphocytes for up to 72 h (ref. 10). We have been able to demonstrate, using a chromium-release technique⁸, that human lymphocytes incubated for several days with irradiated LCL cells show a markedly enhanced capacity for the destruction of these cells (Tables 1 and 2). The cytotoxic effect seemed to require direct cell to cell contact; it was not obtained with supernatants of mixed cultures of irradiated LCL cells and blood lymphocytes.

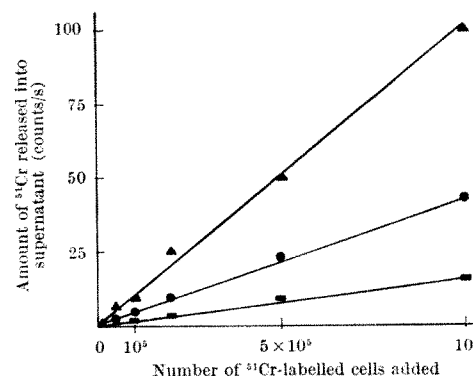


Fig. 1. Amount of ⁵¹Cr released into the supernatant as a function of the number of ⁵¹Cr-labelled EB2 cells added. Human cord blood lymphocytes (2 × 10⁶ in 1 ml.) were incubated for 3 days with or without X-irradiated EB2 cells. 0.5 ml. of medium was removed and 0.5 ml. fresh medium containing the ⁵¹Cr-labelled EB2 cells added. After a further 18 h incubation the supernatants were separated from the cells and counted for ⁵¹Cr. ▲, Cord blood lymphocytes incubated with X-irradiated EB2 cells (10⁵); ●, cord blood lymphocytes without stimulant; ■, red blood cell controls. Points are means of triplicate cultures.

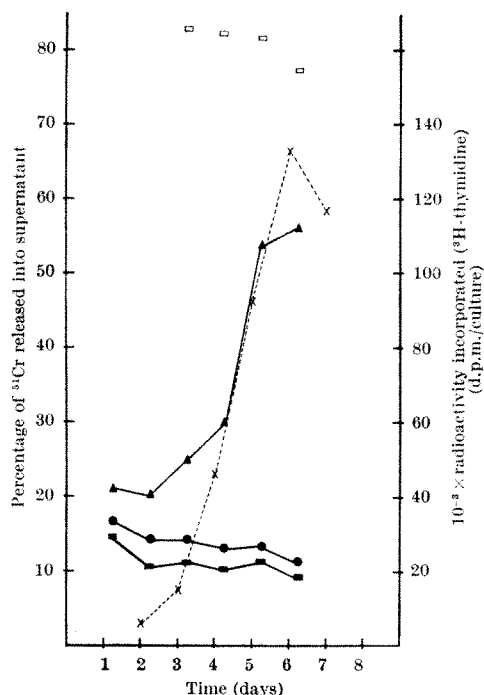


Fig. 2. Cytotoxic effect of adult human lymphocytes stimulated with X-irradiated EB2 cells. Adult human lymphocytes (10^6 in 1 ml.) were cultured with or without X-irradiated EB2 cells. The stimulation was measured by the addition of ^3H -thymidine 24 h before harvesting at the time shown (\times - - \times). The results are expressed as increments ("stimulated" minus controls) and are means of triplicate cultures. To measure cytotoxicity, 0.5 ml. of medium was removed from the cultures without disturbing the cells and 10^6 non-irradiated ^{51}Cr -labelled EB2 cells were added in 0.5 ml. medium. After incubation for 6 h the cultures were centrifuged and the supernatants carefully pipetted off. The cell button was dissolved in 0.1 ml. 1 M NaOH. The cytotoxic effect was measured by the percentage of ^{51}Cr released into the supernatant and the points are means of triplicate cultures. A batch of EB2 cells freshly labelled with ^{51}Cr was used at each time. Δ — Δ , Human lymphocytes incubated with X-irradiated EB2 cells (2×10^5); \bullet — \bullet , human lymphocytes without stimulant; \times — \times , red blood cell controls. Maximum release was measured after two cycles of freezing and thawing of the ^{51}Cr -labelled EB2 cells and is shown for each batch \square .

Nor was there any indication of the need for an antibody or other factor present in human serum, for positive results were obtained when all the cultures contained foetal calf instead of human serum. The cytotoxic effect on the target cells was a rapid process nearing completion in a few hours and apparently beginning as soon as cell contact was established. Killing of the target cells seemed not to result in death of the lymphocytes—that is, it was probably not a suicidal act. These results parallel reports of the effects of immune rat lymphocytes on mouse fibroblasts¹¹ and of immune mouse lymphocytes on a mouse mastocytoma¹².

The reaction was of first order with respect to target cells (Fig. 1), which recalls the first order lytic reaction of

Table 2. EFFECT OF ACTIVATED LYMPHOCYTES ON VARIOUS TARGET CELLS

Composition of culture	Days culture	⁵¹ Cr-Target cell	Percentage ⁵¹ Cr release	
			Test	Control
CB41 (2 × 10 ⁶)	5	EB2	14.7	16.3
		EB4	19.0	16.6
CB41 (2 × 10 ⁶) + X-irrad. EB2 (10 ⁵)	5	EB2	34.3	11.3
		EB4	35.1	15.5
CB41 (2 × 10 ⁶) + X-irrad. EB4 (10 ⁵)	5	EB2	54.3	11.2
		EB4	47.9	15.2
CB40 (2 × 10 ⁶)	7	EB2	12.6	14.4
		Jiyoye	19.6	21.9
CB40 (2 × 10 ⁶) + X-irrad. EB2 (2 × 10 ⁵)	7	EB2	45.4	12.3
		Jiyoye	26.9	19.3
CB40 (2 × 10 ⁶) + X-irrad. Jiyoye (2 × 10 ⁵)	7	EB2	24.6	13.6
		Jiyoye	23.4	20.9
CB37 (2 × 10 ⁶)	6	EB2	16.1	10.3
		L5178Y	18.2	19.7
CB37 (2 × 10 ⁶) + X-irrad. EB2 (2 × 10 ⁵)	6	EB2	49.8	10.7
		L5178Y	30.4	19.1

CB=human cord blood lymphocytes. Other details as for Table 1.

mouse fibroblasts reported by Berke *et al.*¹⁵. Enhanced cytotoxic activity of blood lymphocytes of an adult was first apparent one day after incubation with X-irradiated LCL cells and was maximal at about 6 days (Fig. 2). The increase in cytotoxic activity roughly paralleled the rise in DNA synthetic activity but in other respects there was no correlation between the two activities (see Table 1). The induced cytotoxicity was not specific for a particular LCL, at least as far as EB2, EB4 and Jiyoye LCL were concerned, or even for human cells (Table 2). Mouse lymphoma (L5178Y) cells were lysed as effectively as EB cells by lymphocytes prestimulated with X-irradiated EB cells, even though X-irradiated mouse lymphoma cells are not able to induce significant mitotic activation of human lymphocytes in culture. Human lymphocytes mitotically activated with phytohaemagglutinin or staphylococcal filtrate¹³ did not show enhancement of cytotoxicity by contrast with results obtained with Chang cells as target cells⁹. Unstimulated peripheral lymphocytes from most adult and cord bloods were cytotoxic in some degree for LCL cells but there were quantitative individual variations in primary cytotoxic capability. Lymphocytes from human thymus (obtained at operation through the kindness of Dr B. Bradley) were activated in terms of DNA synthesis by incubation with X-irradiated EB cells but did not acquire cytotoxic competence (Table 3). Thymocytes have also been reported to show poor phytohaemagglutinin-induced cytotoxicity towards Chang cells¹⁴. The effector property may depend on the state of maturation of the thymus lymphocyte or may indicate that more than one cell type cooperates in the effector stage.

Table 3. LACK OF CYTOTOXIC ACTIVITY BY HUMAN THYMUS CELLS

Stimulant added to culture	Percentage ^{51}Cr release		^3H -Thymidine incorporation (d.p.m.)			
	Day 4		Day 3		Day 5	
	Test	Control	Test	Control	Test	Control
None	30	34	754	—	765	—
PHA (2 μg Burroughs Wellcome X5)	30	(34)	1,487	—	4,779	—
Staphylococcal filtrate (0.1 ml.)	26	(34)	470	—	693	—
X-Irrad. EB2 (10^5)	30	(34)	3,384	2,296	17,774	1,366

Human thymus cells (10×10^6 in 1 ml.) were cultured for the times shown, with or without stimulant. The cells were harvested at the time shown. $0.5 \mu\text{Ci}$ ^3H -thymidine was added 24 h previously. To measure the cytotoxic effect, 10^5 ^{51}Cr -labelled non-irradiated EB2 cells were added 24 h before harvesting. Other details as for Table 1.

Most reports of cytotoxic effects of lymphocytes on tumour cells and other cells assume that the cytotoxic reactivity is specifically directed towards the target cells. This is far from proven (see review¹⁴). Human lymphocytes activated by a variety of substances, including phytohaemagglutinin and bacterial products, have been shown to acquire enhanced capacity to kill Chang cells⁹. The cytotoxic specificity of splenic cells from mice immunized with allogeneic tumour cells, on the other hand, seems to be related to the nature of the transplantation antigens on the tumours¹². The conclusions from our own experiments are (a) that cytotoxic activation of lymphocytes is distinct from mitotic activation: both types of activation may occur after exposure to lymphocytes to antigen; (b) the specificity of the effector reaction against target cells is broader and different from that of the mitotic activation. It may also depend on the relative susceptibility of the different cell types to lethal damage.

The whole process of recognition and destruction of cultured LCL cells seems to be an example of a complete reaction of cellular immunity *in vitro* mediated by a single cell type. That this process may be an *in vitro* counterpart of an immunological surveillance mechanism is indicated by recent experiments (ref. 16 and unpublished data) in which similar results were obtained with lymphocytes and LCL cells originating from the same donor.

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Migration of Small Lymphocytes from the Skin to the Regional Lymph Nodes

THE route of sensitization of lymphocytes to homografts has for long been a matter for speculation, and two schools of thought exist. It has been suggested that sensitization occurs either peripherally in the graft¹ or, alternatively, centrally in the regional lymph node^{2,3}.

In the case of the renal homograft, Strober and Gowan have clearly demonstrated that lymphocytes entering the allogeneic kidney are sensitized in the graft and leave again through the venous blood⁴. These cells then settle in lymph nodes and splenic white pulp where they transform into pyroninophilic cells which are so characteristic of the homograft and other cell mediated immunological reactions^{5,6}. Thus, in this particular instance, the evidence for peripheral sensitization is very strong. In the case of the skin homograft the route of sensitization is very much more difficult to demonstrate by direct methods. The chief drawback is the small size of the blood vessels to the graft bed, making them impossible to cannulate. Thus indirect evidence has been sought. Barker and Billingham have clearly demonstrated the importance of the lymphatics of the graft bed⁷. Grafts deprived of the lymphatic drainage enjoy considerably prolonged life, and furthermore do not sensitize the host. Similarly, tissue grafts placed in the hamster cheek pouch, which has no lymphatic drainage, also survive for a long time⁸.

It may be argued, however, that the importance of the afferent lymphatics lies in the fact that they afford a route by means of which antigenic material from the graft may reach the node and so effect a central sensitization. In favour of this alternative to peripheral sensitization, it has been demonstrated that the afferent lymph draining a skin homograft does not contain increased numbers of lymphocytes but does contain appreciable amounts of cellular debris⁹. Barker and Billingham have indicated, however, that if any antigenic cell debris does leave the graft it is not sufficient to sensitize the host⁷.

Furthermore, they showed that in the lymphatic-free skin graft preparation lymph containing lymphocytes did accumulate within the graft.

Any consideration of the route of sensitization must take into account the cellular changes in the lymph node draining a graft. The pyroninophilic reaction that is seen in these nodes is restricted to the diffuse cortical zone (=traffic area), and does not generally involve the germinal centres or medulla until late in the reaction^{6,10}. One route for central sensitization has been advanced which takes this cellular reaction pattern into account. Burwell suggested that the antigen enters the node via the afferent lymphatics and passes from the marginal sinus into the cortex through the post-capillary venules and submarginal capillaries^{2,3}. It is in these vessels that the lymphocytes entering the node in the blood are sensitized. If, however, a "classical" antigen is injected subcutaneously, then this is found to be localized in the medullary sinus cells and germinal centres, and not in the cortical blood vessels or diffuse cortex¹¹. Such classical antigens result in a clearcut plasma cell reaction in the medulla and antibody production¹².

It would considerably strengthen the case for peripheral sensitization if it could be shown that lymphocytes entering the skin (from the blood) can pass down the lymphatics and so reach the lymph node. The experiments described here were designed to test this point, and further to determine where in the node such lymphocytes settle. Do they reach the medulla as particulate material does, or do they show a preference for the diffuse cortical tissue where the homograft reaction occurs?

Peripheral blood lymphocytes were separated from heparinized rat blood (30 IU heparin/ml.) which had been collected by cardiac puncture. The lymphocytes were then concentrated to give a concentration of $20-30 \times 10^6$ /ml. in TC 199 and incubated with ³H-adenosine (10 μ Ci/ml.) for 45 min¹³. The lymphocytes were then washed once with TC 199, and concentrated so that 5×10^6 viable cells were present in 0.1 ml. of TC 199. This procedure labelled approximately 45-50 per cent of the small lymphocytes. Some red blood cells were also present in the inoculum and they acted as a convenient marker for the injection site. 0.1 ml. of the cell suspension was then injected intradermally into the lateral thoracic region of adult male isogenic rats; a clamp was placed over the needle track to prevent leakage of cells out until the needle hole had been sealed with 'Norbecutane'. Animals were killed at varying times up to 48 h, their draining and contralateral axillary nodes, the inguinal nodes and spleen were removed, fixed with Carnoy's fluid or formal saline and 5 micron sections subjected to autoradiography using Ilford L4 nuclear research emulsion. After 14-19 days' exposure at -20°C . the autoradiographs were developed and stained with either methyl-green-pyronin or haematoxylin/eosin.

At least three sections taken from different levels of each node and the spleen were entirely scanned using a $\times 100$ oil immersion objective. The numbers of cells seen in the marginal sinus, lymphoid follicles, diffuse cortical tissue and the medulla of the node were noted. Sections made through the skin in the area of the injection were also treated in the same way. As a control experiment lymphocytes were labelled and then killed by heating at 56°C for 20 min. After this treatment an average of 98 per cent of the cells were dead and yet still intact and labelled.

The results obtained were very consistent in each of the three animals killed at each time interval. Examination of lymph node sections from serially killed rats showed that during the 15 h following injection no labelled cells had reached any of the draining lymph nodes. In the sections of the injection site most labelled cells were seen amongst the injected red cell mass with very few labelled cells in the dermis. From the sixth hour onwards, however, labelled cells left the injection site and were seen in increasing numbers throughout the dermis. At the

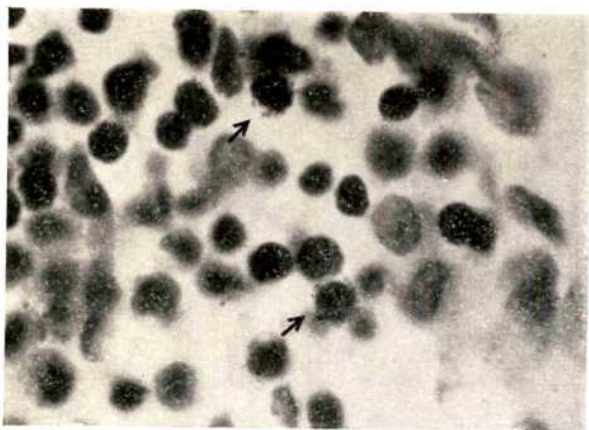


Fig. 1. ^3H labelled lymphocytes in the marginal sinus of regional lymph node 21 h after injection (haematoxylin and eosin, $\times 800$).

injection site there was a vigorous polymorph infiltration and a few histocytes were also present.

In the draining nodes a few labelled cells were first found in the animals killed at 15 h. These cells were only seen in the marginal sinuses and occasionally in the cortical area immediately adjacent to that sinus. By 20 h, however, the number of labelled cells in the draining node had increased considerably and they were distributed through all zones of the node except in the lymphoid follicles and germinal centres when they were present. The majority of cells were, however, found in the diffuse cortical tissue (61 per cent) but significant numbers were also seen in the marginal sinus (30 per cent) and the cords of the medulla (9 per cent). By 24 h the progressive movement of the labelled cells across the node had continued and many more cells were seen in the diffuse cortex (88 per cent) with a few still in the marginal sinus (3 per cent), but little change in the number of cells in the medulla (9 per cent) some of which were now, however, in the medullary sinuses (see Figs. 1, 2 and 3).

In both 20 and 24 h regional lymph nodes, labelled lymphocytes were seen in the cortex near to, and in, the intermediary sinuses, and some labelled cells were seen in these sinus walls. Other labelled cells were seen in the proximity of post capillary venules, although no labelled cells were found in the lumen of these vessels. The distribution of the labelled cells was not uniformly spread throughout the diffuse cortical tissue, but had a segmental distribution. This segmental distribution also occurs if the cells are injected intravenously¹³ and may account for the segmental pattern of reaction in nodes—draining a homograft². Some radioactivity had been injected with the cells, and this gave rise to a light labelling in some of the macrophages of the marginal sinus and medulla (Fig. 3). This light degree of macrophage labelling was also seen in nodes examined 6 h after injection of the cells into the skin. A second possibility is that the light labelling may have been caused by label released from dead lymphocytes after their ingestion and destruction by the macrophages. This is, however, less likely.

By 48 h very few labelled cells were seen in the draining node in two out of the three animals, although in the third animal a significant number of labelled lymphocytes was found. The localization of these cells in the node was still, however, chiefly in the diffuse cortex (76 per cent) and medullary cords and sinuses (24 per cent). No cells were found in the marginal sinus. In another group of animals the labelled cells were injected into the tail. In these rats labelled cells were seen at 48 h in all instances in the draining inguinal nodes only with essentially the same distribution in the diffuse cortex (89 per cent) and medulla (11 per cent). A small number of the labelled cells which were seen in the cortex of these 48 h draining nodes

possessed a nucleolus and had cytoplasm with increased pyroninophilia. These cells could be considered to be activated lymphocytes, although the activating stimulus is not known.

Labelled cells were seen in very small numbers in the contralateral lymph nodes from 20 h onwards in most rats. The topographical localization of these cells was identical to those in the draining nodes. No labelled lymphocytes could be found in the more distant inguinal nodes or spleen.

In six control rats injected with labelled dead lymphocytes, there was a prominent histocyte reaction at the injection site by 24 h. Very few intact labelled cells were seen and these were all present in the needle tract and none had penetrated the dermis. No labelled cells were found in the draining, contralateral or distant nodes or in the spleen in these animals. Light labelling of lymph node macrophages was seen in this series also.

Summarizing these results, it may be said that lymphocytes introduced into skin gain access to the draining lymph node and localize in the diffuse cortical area or "traffic area". This is precisely the area in which the pyroninophilic reaction is found in response to a homograft. These cells must have reached the draining node through the afferent lymphatic vessels. They enter the marginal sinuses and can either pass down the intermediary sinuses and then migrate out of these sinuses into the diffuse cortical tissue, or else cross the walls of the marginal sinus and enter the cortex directly. Here they can remain for at least 24 h. This is an active process which requires viable cells. A number of the cells eventually enter the medullary sinuses and probably leave the node in the efferent lymph. The labelled cells cannot have reached the node through the blood stream for two reasons. First, the localization of the labelled cells in the lymph nodes was strictly regional and confined to the draining node; it is difficult to see how this could occur if the labelled cells had returned to the blood after injection into the skin. Second, it has been shown that intravenously injected labelled lymphocytes are found in all nodes and also the spleen and are rarely found in the marginal sinus or in the area of cortex immediately under it¹³.

Thus these experiments have demonstrated a second route of lymphocyte circulation from connective tissue through the lymphatics to the lymph nodes. Lymphocytes are ubiquitous in the connective tissues of the body, and can be shown to be leaving the skin continuously through afferent lymphatic vessels². Unless it is to be postulated that lymphocytes are produced in the connective tissue of the skin, for which there is no good evidence, then these cells must enter the tissue from the blood. As in the case of the blood→lymph node→lymph→blood recirculation¹³, the area of the node in which the migratory

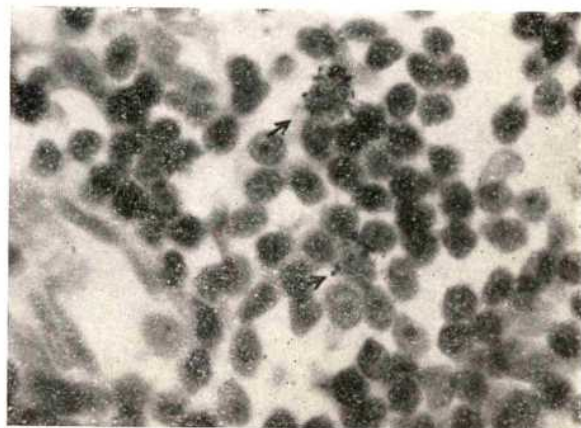


Fig. 2. ^3H labelled lymphocytes in diffuse cortical tissue 21 h after injection (haematoxylin and eosin, $\times 560$).

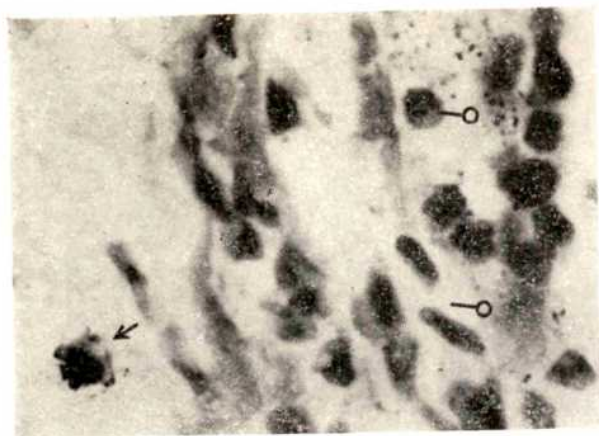


Fig. 3. ^3H labelled lymphocyte in medullary sinus (\leftarrow) and lightly labelled sinus macrophages (\circ) in regional node 24 h after injection (haematoxylin and eosin, $\times 800$).

lymphocytes accumulate is the diffuse cortical zone. Such a connective tissue-to-node route was suggested by Sjövall in 1936¹⁴, but was discounted by Yoffey and Drinker as a major recirculation route because of the low number of lymphocytes present in peripheral lymph¹⁵. It is submitted, however, that although this route is not the major route of lymphocyte circulation it does become of paramount importance in the context of the homograft reaction. Using the data of Hall⁹ it may be calculated that lymphocytes (and other cells) reach the node through an afferent lymphatic at a rate of up to 3×10^6 per hour which could lead to considerable accumulation of cells in the node over a period of a few days.

Previous *in vitro* studies by me¹⁶ and others¹⁷ have shown that lymphocytes can be sensitized to foreign transplantation antigen by contact with homologous cells. Moreover, studies of the mixed leucocyte reaction have shown clearly that the result of this sensitization is the production of a population of pyroninophilic cells capable of mitosis which are very similar in their ultra-structure to many of the pyroninophilic cells found in the lymph node draining a homograft. The observations reported here taken together with these *in vitro* studies, and the demonstration of the importance of the lymphatics in sensitization to skin graft, make a very strong case for peripheral sensitization in the case of the skin graft, as well as the renal homograft.

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Postsynaptic Action of Cobra Toxin at the Myoneural Junction

RECENT investigations of peptide fractions from cobra venoms suggest that the most potent acute toxicity arises from a curare-like block of the action of acetylcholine at the skeletal myoneural junction¹⁻³. I wish to report the effects on myoneural transmission of toxin T_3 from *Naja naja siamensis*—(Kaouthia) (Thailand cobra) venom, a pure peptide of known amino-acid composition⁴.

I used standard intracellular recording techniques at room temperature (18° – 22° C) on superficial fibres of the isolated frog (*Rana pipiens*) sartorius myoneural junction preparation⁵, with special attention to favourable signal-to-noise ratios. The nerve was stimulated at a frequency of 0.5/s at 5–10 min intervals. I used digital computers (Control Data 160-A and 160-G) for data collection, measurement, and statistical analysis. The Ringer solution contained 113 mM NaCl, 2.5 mM KCl, 4 mM MgCl_2 , 5 mM Tris (pH 7.3), 0.4 mM Ca^{2+} , and 4 mM Mg^{2+} . The concentration of toxin was 1.6×10^{-6} M.

With these divalent cation concentrations, end plate potentials (EPPs) consist of several summed miniature EPPs, and EPP amplitudes have a Poisson distribution⁶. I determined m , the average number of miniature EPPs comprising the EPP, in three ways from the measurements of EPPs and of miniature EPPs⁷.

Fig. 1 shows that after the addition of toxin, the EPP decreased and eventually attained an exponential rate

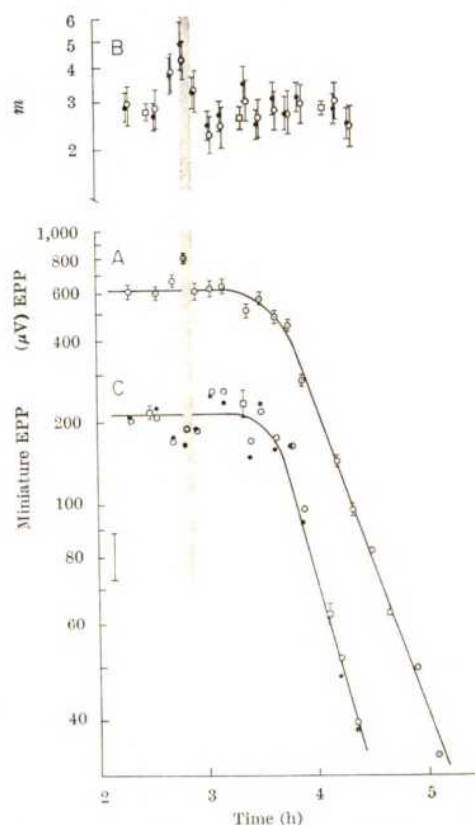


Fig. 1. Action of a pure peptide from cobra venom on myoneural transmission in the frog. At cross-hatching Ringer solution was replaced by solution containing toxin. \circ , \bullet and \square . Measurements on a series of 128 EPPs. \square , Measurements on spontaneously occurring miniature EPPs. A, Average EPP amplitude \pm s.e. EPPs without error flags at later times were too small, relative to the recording noise, for accurate measurements on individual responses. B, Quantal content $m \pm$ s.e. \circ , m , computed from the number of failures; \bullet , m , computed from the coefficient of variation of the EPP amplitude distribution; \square , m , computed by dividing EPP by miniature EPP amplitudes. C, Miniature EPP amplitude: \square , measured directly; \circ , computed as EPP/m ; \bullet , computed as EPP/m . The vertical line at the lower left gives approximate error limits for computed miniature EPPs.

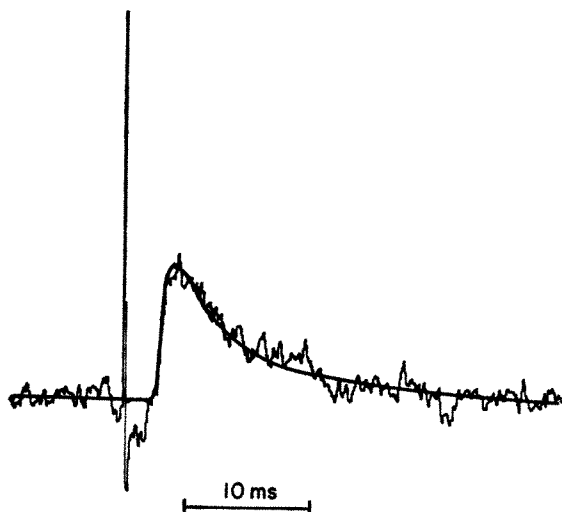


Fig. 2. Waveforms of 128 averaged EPPs, before (smooth, heavy trace) and 150 min after (light, noisy trace) treatment with cobra toxin as described in the text. Waveforms have been amplified to match peak amplitudes; actual amplitudes were 2.1 mV before, 25 μ V after. Amplifier was capacitance-coupled with a time constant of 100 ms.

of decline. This time course indicates that the toxin acted irreversibly in these experimental conditions; washing with Ringer (not shown) halted the decline but caused only slight recovery after several hours. The lines *A* and *C* are parallel on semilogarithmic coordinates, indicating that EPP amplitude decreases because miniature EPP amplitude decreases; *m* remains unaffected. I have also found that the toxin blocks the response to micro-ionophoretic applications of acetylcholine and of carbachol (see also ref. 1). Thus, the toxin decreases the postsynaptic sensitivity to acetylcholine.

Fig. 2 compares the time course of averaged EPPs before and after application of the toxin. The waveforms superimpose: therefore, the toxin does not affect conduction in the nerve terminal, passive electrical properties of the muscle membrane, or the acetylcholinesterase. The toxin altered neither the spontaneous miniature EPP frequency nor the muscle fibre resting potential.

Some experiments showed a decline in *m* in addition to the decrease in miniature EPP amplitude. In these anomalous cases, the toxin usually increased the coefficient of variation of miniature EPP amplitudes while it decreased the average miniature EPP amplitude. This leads to a broader EPP amplitude distribution, therefore to a lower m_2 . Also, the broader miniature EPP distribution probably allowed the smallest miniature EPPs to escape detection, thus increasing the average miniature EPP amplitude and decreasing m_1 . In similar fashion, EPPs consisting of only one small miniature EPP probably were recorded as failures, thus decreasing m_0 . The broader miniature EPP distribution could arise from local variations in the accessibility of postsynaptic membrane to toxin molecules, because of variations in the connective tissue, Schwann cells and basement membrane covering the end-plate⁸. Also, some branches of the end-plate may lie on the surface of the muscle while others lie in the cleft between adjacent fibres⁹. Such mechanisms are likely because the time to reach an exponential decline, and the normalized rate of decline, varied by a factor of 3 even when two end-plates a few hundred μ m apart were monitored simultaneously.

Toxin *T_3* therefore acts specifically with low reversibility on the acetylcholine receptor at the skeletal myoneural junction. Homologous peptides occur in other elapid and hydropheid venoms; I have obtained similar but less extensive results with *Naja nigricollis* toxin α , the sequence of which is known¹⁰. It is still not known whether these toxins bind as specifically as they act.

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Intracellular Responses to Light from Cat Pigment Epithelium: Origin of the Electroretinogram c-Wave

NOELL¹ first showed that the c-wave of the vertebrate electroretinogram (ERG) probably originates in the pigment epithelium because the c-wave disappeared in the rabbit after selective poisoning of the pigment epithelium by sodium iodate. Brown and Wiesel showed that the c-wave of the cat's local ERG attained maximum amplitude when the electrode was immediately adjacent to the pigment epithelium². They also obtained more direct evidence during brief intracellular recordings from pigment epithelial cells, which revealed larger potentials of similar time course but opposite polarity to the c-wave³. We have now obtained stable intracellular recordings which permit the slow light-evoked responses of pigment epithelial cells to be described in detail and identified positively as the origin of the c-wave.

The preparation and maintenance of the cat, and methods of stimulating and recording from the retina of the intact eye, have been described elsewhere^{3,4}. All responses were recorded from the area centralis after dark-adapting the retina at least 45 min. Recordings were made by glass micropipettes filled with 3 M KCl and a reference electrode on the temporalis muscle. The frequency response of the recording system was flat from 0–2,500 Hz.

The pigment epithelium was located by advancing the microelectrode to the retinal depth where the c-wave of the local ERG attained maximum amplitude. A slight additional advance penetrated a mechanical barrier, accompanied by a negative baseline shift of at least 20 mV and a marked diminution of the entire local ERG². Intracellular responses were sometimes recorded while passing through the barrier, but were more often obtained by penetrating the barrier and then withdrawing in 1 μ m steps until the baseline showed a negative shift of 50–60 mV. When such a resting membrane potential was recorded a characteristic slow negative response to light always appeared (Fig. 1).

Brown and Tasaki have shown by electrode marking that after penetrating the described barrier the electrode is located against the choroidal side of Bruch's membrane, while withdrawal through the barrier to the site of maximum c-wave amplitude places the electrode against the retinal side of the pigment epithelium⁵. Our intracellular recordings could only have been obtained from pigment epithelial cells, for they are the only cells between the two surfaces of this mechanical barrier. Numerous attempts were made to identify the recording site by

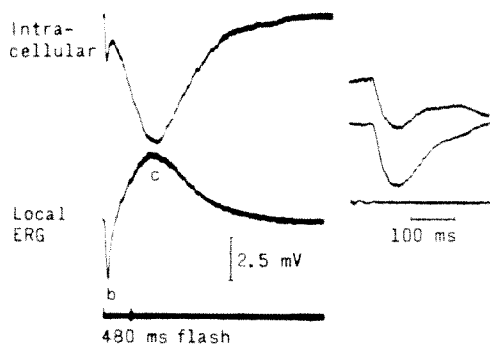


Fig. 1. Comparison of the local ERG and an intracellular response from a pigment epithelial cell. The intracellular response was recorded first; then the microelectrode tip was withdrawn into the photoreceptor layer and the ERG was recorded to an identical flash. The records on the right were photographed at a faster sweep to illustrate the similar time courses of the *b*-wave of the local ERG (bottom) and the early peak of the intracellular response (top). The responses were obtained in a dark-adapted retina with a 440 nm filter, stimulus intensity of 2.0 log *td* scotopic, stimulus spot diameter of 2.0 mm, and a repetition rate of one per 5 s. Negative responses are displayed downward, in this and subsequent figures, in accord with the conventions for both intracellular recording and ERG work.

electrophoresis through the microelectrode of Procion Yellow M4RS and Niagara Sky Blue 6B during intracellular recording. Although this technique succeeded readily with horizontal cells⁶, dye marks deposited in pigment epithelial cells during the same experiments were never recovered. Because these cells are specialized for exchanging substances between the retina and choroid⁷, the dye may have been lost into the choroidal circulation; also it may have diffused into adjacent pigment epithelial cells through tight junctions^{8,9}.

Intracellular responses obtained in thirty-three impalements were maintained stably from 3–40 min. Fig. 1 shows an intracellular response compared with the extracellular response (local ERG) elicited by an identical flash after withdrawing the microelectrode tip back into the photoreceptor layer. The intracellular response was always negative (hyperpolarizing) and in the best recordings reached a maximum amplitude of 20 mV superimposed upon a resting potential of 60 mV. The slowness of this response is its most distinctive feature, distinguishing it from all other potentials evoked by light from the normal mammalian retina. The time constant of the rise averaged 800 ms (775–850), while the time constant of the fall was nearly the same, about 750 ms. The identity of the slow intracellular response with the *c*-wave is indicated by their similar time courses, including rise, peak, and fall times. The slow intracellular response was always opposite in polarity from the *c*-wave, and larger than the *c*-wave, as expected if the *c*-wave is generated across the apical membrane of the pigment epithelium.

The identity of the intracellular slow potential and the *c*-wave is strongly supported by their similar response to

changes in stimulus parameters. Fig. 2 shows effects of stimulus duration upon both the intracellular slow potential and the *c*-wave. At short stimulus durations the intracellular response peaks after termination of the stimulus. As stimulus duration increases, response amplitude increases until a plateau is reached which is only prolonged by still longer stimuli. Similar effects occur in the *c*-wave of the local ERG, which for any given stimulus duration follows closely the time course of the intracellular response. When stimulus intensity was increased, the amplitude of the intracellular slow potential increased and the response peak occurred at progressively longer delays after termination of the stimulus. Here also, corresponding effects occurred in the *c*-wave recorded after withdrawing the microelectrode into the photoreceptor layer.

Intracellular responses also exhibited an early negative peak on the rising phase of the slow potential. The records at a faster sweep speed, on the right side of Fig. 1, show that this peak has the same latency and time course as the *b*-wave of the local ERG. Because these two responses have the same polarity, and the *b*-wave is known to be generated more proximally in the retina², we concluded that the intracellular negative peak is not generated across the apical membrane of the pigment epithelium, but represents intracellular detection of an extracellular potential, as found in glial cells¹⁰.

Spectral sensitivity functions for the intracellular slow potential under dark-adapted conditions are shown in Fig. 3. The curves obtained closely resemble rod sensitivity functions of cat S-potentials. No Purkinje shift occurred when the criterion voltage for plotting spectral sensitivity was raised from 2.5 to 5.0 or 7.5 mV, although flashes were sufficiently intense to excite cones, particularly at the long wavelengths. In addition, there was no Purkinje shift after light-adapting the retina by intense flashes, and recovery of the response after light adaptation was very slow. In short, a cone contribution was sought but not found, although the recordings were in the area centralis where the cone population is densest and where most S-potentials are influenced by cones as well as rods^{4,11}. It seems, therefore, that the intracellular slow potential depends upon absorption of light by rhodopsin. This result also agrees well with studies of the *c*-wave, which is absent in pure cone retinas^{12–14} (with one apparent exception¹⁵), disappears in light-adapted mixed retinas¹⁶, and follows a rod spectral sensitivity function^{17,18}. Although rhodopsin has been identified in pigment epithelial cells¹⁹, two observations indicate that light which triggers the *c*-wave is absorbed in the rod outer segments. The *c*-wave has been reported absent from isolated pigment epithelium-choroid preparations of cold-blooded eyes²⁰, but the pigment epithelium may be damaged in such cases. Recently a more critical result was obtained by Liebman *et al.*¹⁹, who showed that the action spectrum of the *c*-wave is not modified by pre-absorption in rod rhodopsin in the

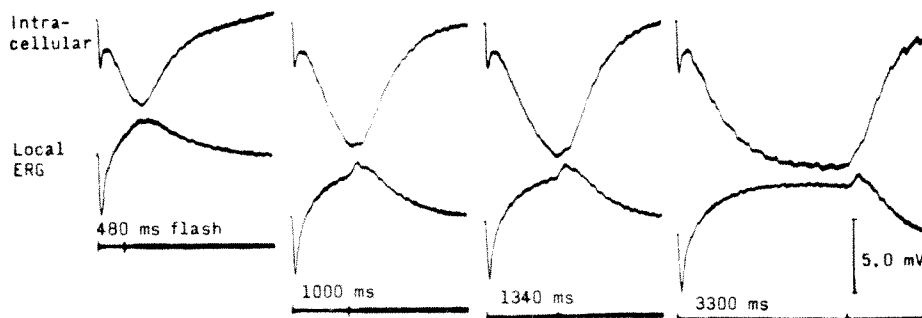


Fig. 2. Comparison of the local ERG and the intracellular response of a pigment epithelial cell as a function of stimulus duration. Intracellular responses were recorded first; then the microelectrode tip was withdrawn into the photoreceptor layer and local ERGs were elicited by identical stimuli. The responses were obtained in a dark-adapted retina with "white" stimulus flashes (colour temperature 2,850 K) at an intensity of 1.7 log *td* scotopic. The stimulus spot was 2.0 mm in diameter and the repetition rate was one per 5 s.

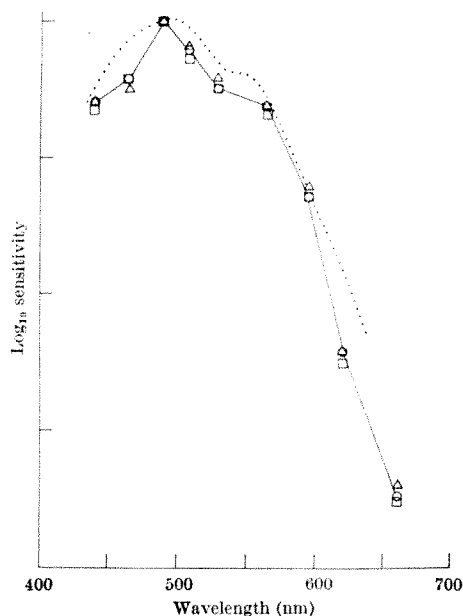


Fig. 3. Spectral sensitivity of the intracellular response of a pigment epithelial cell in a dark-adapted retina. Responses to increasing stimulus intensity were obtained at different wavelength maxima spaced from 440 to 660 nm. The stimulus was 2.0 mm in diameter, 480 ms in duration, and was repeated every 5 s. Responses were measured from baseline to peak. Spectral sensitivity curves were then derived by applying constant response criteria to the amplitude-intensity functions. The relative sensitivity values at the criteria were then converted to spectra of equal quantum intensities. Criterion voltage: ○, 2.5 mV; □, 5.0 mV; Δ, 7.5 mV. The solid line was drawn through data points for the 2.5 mV criterion of response amplitude. The spectral sensitivity of dark-adapted cat S-potentials is also shown for comparison (dotted line)⁴.

manner expected if the rhodopsin which initiates the *c*-wave were contained in the pigment epithelium.

The chief remaining problems concern how rod excitation initiates an electrical response across the apical membrane of the pigment epithelial cell, the functional significance of this response, and why it is not similarly initiated by cone excitation. Electrical interaction of excited rods with pigment epithelial cells seems unlikely, for tight junctions have not been found between these cells²¹⁻²³, and because the 10-20 nm extracellular space should effectively uncouple the membranes from electrical interaction²⁴. An important key seems to be the slowness of the response generated across the pigment epithelial membrane. The faster rise time of the rod component of cat S-potentials⁴ indicates that even if the output signal of the rod receptor acts as an intermediate in initiating the slow potential of the pigment cell, it could account for only a fraction of the slowness of that response. Diffusion across the extracellular space will also play only a small part unless the molecule is very large or the interstitial matrix acts as a diffusion barrier^{24,25}. It seems likely, therefore, that the ion or molecule that diffuses between the two cells and initiates the slow potential is generated slowly, as would be the case with a late photoproduct.

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Fragmentation of a Geometrical Figure viewed under Intermittent Illumination

WHEN a simple target is viewed as a currently occurring retinal image which is confined to a specific locus, the target has been reported to lose contrast and to disappear¹⁻³. The restriction of an image to a specific retinal locus is commonly termed a "stabilized retinal image". This is the reverse situation to that encountered in normal vision where eye movements change the position of the retinal image several times a second. For more complex targets such as geometrical figures viewed as stabilized retinal images, intermittent disappearance and reappearance of the figure in whole or in part have been reported⁴⁻⁷. This striking phenomenon, usually outside the subject's previous visual experience, has been termed "fragmentation". The common methods of investigating fragmentation are voluntary steady fixation, viewing as a partially stabilized retinal image using a contact lens and as a prolonged after-image (a completely stabilized retinal event)⁴⁻⁷. These methods in order progressively impose restraint on the image with respect to its retinal locus. Another method of investigating fragmentation was described by Evans⁸ and McFarland⁹, who note reports of fragmentation by subjects viewing geometrical figures for brief time intervals. Typically, the stimulus is viewed tachistoscopically near the recognition threshold after which the subject records his observations. This sequence is then repeated for many trials. We wish to describe another method of producing and studying fragmentation, by viewing a geometrical figure under intermittent illumination.

Eight subjects viewed the geometrical figure shown in Fig. 1, in a range of frequencies of intermittent illumination. They were instructed steadily to fixate the centre of the figure and to make verbal and drawn reports of fragmentation, while the frequency of intermittent illumination was slowly increased from 1 to 30 c.p.s. and then



Fig. 1. Stimulus pattern used in this investigation.



Fig. 2. Typical patterns of fragmentation reported by subjects viewing Fig. 1, under intermittent illumination.

decreased again to 1 c.p.s. The frequency of intermittent illumination was held constant while subjects made their reports. Typically, a complete trial lasted 8 to 10 min. Intermittent illumination was produced by placing the outline figure shown in Fig. 1 before a General Radio Company 'Strobotac' type 1531-AB, operating with a Mazda FA/7/s flash tube (standard fitment). The spectral output of this source was continuous within the visual spectrum. Flash duration was rated at 3 μ s for frequencies from 2 c.p.s. to 11.5 c.p.s., and at 1.2 μ s for frequencies above 11.5 c.p.s., power rating at 0.35 W. The geometrical figure was drawn in black Indian ink on white translucent paper. To ensure an evenly illuminated stimulus, a sheet of white translucent plastic was placed between the geometrical figure and the strobatorch. The diameter of the circle subtended a visual angle of 3° and the line width of 7 min of arc, when viewed at optical infinity. A condition of optical infinity was produced by instructing subjects to wear their corrective lenses if normally worn for distance and to view the geometrical figure through a convex lens of focal length 50 cm. The plane of the stimulus was situated in the focal plane of the lens. Viewing was monocular, the non-viewing eye being occluded.

The only illumination present during the experiment was that provided by the strobatorch. All subjects were experienced observers and had prior knowledge of the phenomenon. To minimize any unpleasant effects which might have been induced by the intermittent illumination, the visual field was restricted to that immediately surrounding the geometrical figure. This was achieved by placing a circular aperture in the stimulus plane. At optical infinity the diameter of the aperture subtended a visual angle of 11½°. The stimulus was centrally placed with respect to the aperture.

All subjects reported fragmentation of the stimulus. Some typical observations are shown in Fig. 2. These observations are consistent with those reported when usual methods of producing retinal image stabilization were used. All subjects also reported distortion of the figure, a finding also consistent with previous reports¹⁰. Although there seem to be qualitative similarities of fragmentation between this method and those described previously, it remains to be seen whether there are also quantitative similarities. Strong qualitative and quantitative similarities have already been demonstrated for geometrical figures viewed by steady fixation, with a contact lens and as a prolonged after-image^{6,7,11,12}. Subjects' qualitative observations showed that generally fragmentation occurred between 2 and 16 c.p.s. But there were strong indications from subjects' reports that more fragmentation took place between 6 and 10 c.p.s. than at other frequencies.

This possibility was investigated. Four subjects who had previously reported fragmentation qualitatively reported the occurrence and duration of fragmentation by pressing a button. These responses were recorded on an Esterline-Angus events recorder. The stimulus conditions were those already outlined, except that subjects viewed the geometrical figure at six specific frequencies of intermittent illumination: 3, 6, 9, 12, 15 and 40 c.p.s. Subjects reported fragmentation for 3 min at each frequency. Frequencies were chosen to cover the range within which fragmentation had been reported to occur, qualitatively. A frequency of 40 c.p.s. was chosen because, being well above each subject's C.F.F. for central vision, it simulated a steady fixation viewing condition. The results are shown in Table 1.

Table 1. TOTAL DURATION OF FRAGMENTATION REPORTED BY FOUR SUBJECTS AT FREQUENCIES OF INTERMITTENT ILLUMINATION OF 3, 6, 9, 12, 15 AND 40 C.P.S.

Subject	3	6	9	12	15	40
1	75.0	109.0	154.75	60.0	53.25	16.50
2	3.75	13.5	16.25	17.50	7.0	3.75
3	1.75	84.25	167.50	8.75	1.25	0.00
4	24.75	128.50	171.0	86.0	44.50	45.25

Figures are given in seconds. Total viewing time at each frequency for each subject was 180 s.

Table 1 shows the total time for which each subject reported fragmentation occurring at each frequency of presentation. For three subjects the amount of fragmentation was frequency dependent, that is, it occurred maximally at 9 c.p.s., and took up progressively less of the viewing time at greater or smaller frequencies. One subject reported a similar amount of fragmentation at all frequencies of presentation.

In summary, it can be said that viewing a geometrical figure in conditions of intermittent illumination provides a new method of producing fragmentation. Because these preliminary findings indicate that the amount of fragmentation is dependent on the frequency of presentation, this method may provide a means of investigating temporal factors underlying the phenomenon.

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Mislocation of Test Flashes during Saccadic Image Displacements

If a stationary source of light is flashed briefly during or just before a saccadic eye movement, it is less easily detected than when the eye is stationary¹⁻³, and its location relative to a fixed background is misperceived^{4,5}. These phenomena have sometimes been taken to support the theory that an efferent signal from the oculomotor system actively modifies the visual input signal during a voluntary eye movement so as to 'cancel' the visual effects of the movement⁶, with a view to maintaining the stability of the perceived world. On this theory one would not expect to observe such perceptual anomalies if the eye were held stationary and the retinal image moved in saccadic fashion by external means.

I have reported recently^{7,8} that the threshold for perception of faint flashes can be elevated to about the same extent by passive as by active image displacement. A further series of experiments using the same apparatus have now confirmed that illusory displacement of a flash also occurs in relation to a saccadically displaced field in the absence of oculomotor activity. These experiments have made it possible to study the effects of the time interval between flash and image displacement, and of the speed of displacement, on the magnitude of the illusion.

A horizontal luminous scale subtending 11° was used as the moving field. Its image (white on a dark ground) was projected with a luminance of 1 log foot Lambert on a vertical screen 1 m from the observer in a dark room. It could be rapidly displaced horizontally up to 4° in either direction by means of a mirror mounted on an electroencephalogram (EEG) pen motor. The displacement, which was free of overshoot, could be set to occupy 8 ms or 40 ms, and took place at randomly varied intervals in the neighbourhood of 4 s, the return stroke coming 2 s later. A triangular arrow, which could be illuminated by an electronic flash tube, was arranged so that its image was deflected with the scale and always coincided objectively with the central scale division.

The timing of the flashes was varied randomly in a range of either 100 or 200 ms about the time of onset of the field displacement, and the subject was asked to report the position on the scale that the flash appeared to occupy. To eliminate cues from a stationary fixation point, the subject was instructed to fixate the central scale division in its initial position. Any reflex response to scale movement would have a latency of the order of 100 ms, and so could be discounted⁹.

Typical results are shown in Fig. 1, in which perceived flash location is plotted against the time between flash and onset of field displacement. It is clear that mislocation is confined chiefly to flashes presented in the 50 ms or so before and during displacement, the error curve rising to a maximum at time of onset. Field displacements to left and right produce illusory shifts of similar magnitude in opposite directions.

The effect of increasing the time of displacement from 8 ms to 40 ms is shown in Fig. 2. At the slower speed both the magnitude and time range of illusory displacement were considerably diminished. The position of the peak was not affected, although the "overshoot" error in the opposite sense reached its maximum later—that is at the end of the displacement. Not all subjects experienced this "overshoot".

An interesting anomaly was reported spontaneously by some observers. The flash (which was practically instantaneous) sometimes appeared to have two locations in succession, as if onset and offset happened at different times. Where this ambiguity arose, the subject was asked to report the "earlier" perceived location. Successive "ghost" images in response to transient stimulation are not uncommon¹³, and are perhaps attributable to the oscillatory impulse-response of the visual system¹⁴. The same explanation may account for the "paradoxical double localization" occasionally found by Bischof and Kramer⁵ for flashes presented during saccadic eye movements.

Localization errors when a test line was briefly illuminated against a moving background have been reported briefly by Sperling and Speelman¹⁰, who gave no figures,

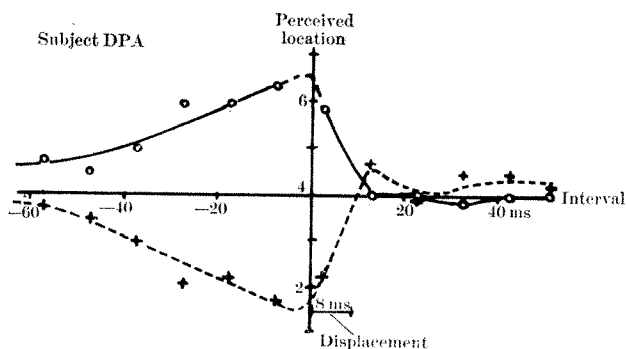


Fig. 1. Perceived location of flash on scale as a function of time interval between flash and onset of displacement. Actual location was fixed at 4. Displacement took 8 ms. Measured displacement to L was slightly larger (2.8 divisions) than to R (2.4 divisions). (○) Left; (+) right.

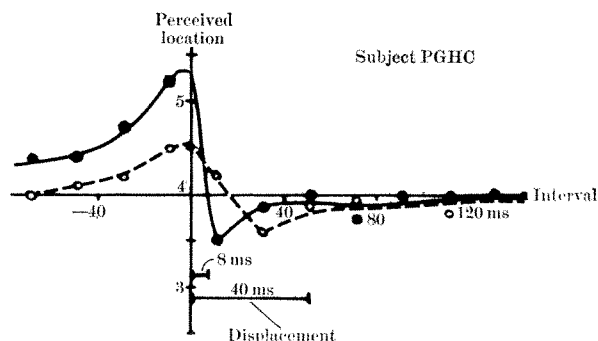


Fig. 2. Effects of duration of image motion on perceived location. (●) 8 ms (500°/s); (○) 40 ms (100°/s).

but stated that "localization during eye movement usually can be predicted from localization during object movement". Taken together with my results (especially Fig. 2), this suggests that some correlation might be expected between the magnitude of the illusory displacement observed with saccadic eye movements and the speed of these movements. In any event, although my results do not conclusively exonerate the oculomotor system, they show that it need not be implicated in order to account for mislocations during saccadic movement. I have argued elsewhere that visual stability during eye movements does not require a "cancellation" mechanism¹¹.

How then are such mislocations to be explained? The clue seems to be the finding (confirmed in a further series of experiments on fifteen subjects with the assistance of Mr K. Bradshaw) that the most significant errors are confined to the period before and during displacement of the image. The inference seems to be that the location of a flashed image relative to its background involves an interaction between the neural signals generated by each, which interaction takes an appreciable time to complete. If during this time the retinal image of the background shifts to a new position, the integrative process will have two different "background" signals to cope with, each making its own contribution to the total weight of evidence with respect to flash location. The later the flash comes, before the moment of transition, the greater will be the weight attached to the new scale-position as compared with the old. Thus the greater will be the illusory displacement of the flash, which is then objectively at the centre of the old scale-position. When the flash is delayed until after the scale-displacement, it is objectively at the centre of the new scale-position, so that only the residue of the earlier background signal remains to contribute any bias to the computation of relative flash location. This bias would account for the "overshoot" observed with some subjects.

If this explanation is valid, the shape of the error curves in Figs. 1 and 2 should be closely related to the time course of the postulated interaction. The data suggest a duration of the order of 50 ms for the integrative process, which is comparable with that inferred for the flash-detection process in the earlier experiments^{7,8}. Retinal ganglion cell discharges of about this duration are typical in response to brief threshold stimuli¹². The correspondence is at least encouraging; and the strong dependence of the magnitude of the illusion on the duration of the movement strengthens the hypothesis that it depends essentially on the degree of overlap in time between the neural events representing the flash and those representing the scale in its successive positions.

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Test of Gregory's Constancy Scaling Explanation of the Müller-Lyer Illusion

MUCH of the recent interest in geometrical illusions has centred on a size constancy explanation by Gregory¹⁻³. Size constancy occurs when the apparent size of an object remains constant, independent of the distance of the object from the observer, even though the size of the retinal image varies with the distance. The mechanism which produces size constancy has been called constancy scaling².

In Gregory's theory—a modification of perspective theory⁴—geometrical illusion figures are considered to be similar to two-dimensional projections of three-dimensional figures. As in three dimensions, constancy scaling operates to increase the apparent size of apparently more distant parts of a figure, and to decrease the apparent size of apparently nearer parts. In a two-dimensional figure, perspective features provide the observer with cues for judging the apparent distance of parts of the figure. These perspective features trigger constancy scaling, which affects the apparent size of parts of the figure and produces an illusion, even though the figure need not be seen as having depth.

Gregory's theory cannot account for all illusions because, as Day⁵ has pointed out, illusions can be generated by figures without perspective features, such as the dumbbell version of the Müller-Lyer figures. It is still, however, of interest whether illusions in figures containing perspective features can be explained by the operation of constancy scaling. The Müller-Lyer figures seem to have perspective features: the wings and their directions. If Gregory's theory is correct, constancy scaling should thus influence the perception of the Müller-Lyer figures.

Gregory² assumes that Müller-Lyer figures are similar to projections of typical three-dimensional objects. For example, Fig. 1a—with the wings directed outwards—is similar to a projection of the corner of a room, where the central axis corresponds to the corner and the outside wings correspond to the intersections of the walls with

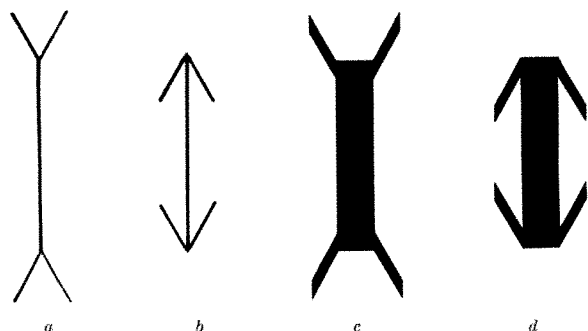


Fig. 1. a and b, Müller-Lyer figures. c and d, The same figures with enlarged width of central axis.

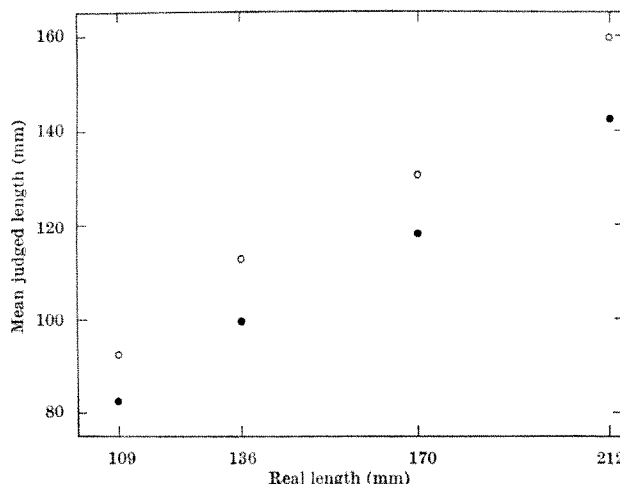


Fig. 2. Mean judged length of the central axes of Müller-Lyer figures as a function of the absolute length and the direction of the wings (●, inward; ○, outward).

the ceiling and with the floor. Because of the effect of the wings as perspective cues, the central axis is judged as though it were farther away than the wings, that is, as though the figure were a three-dimensional object. Accordingly, constancy scaling operates, and increases the apparent length of the central axis. On the other hand, Fig. 1b—with the wings directed inwards—has the perspective cues of a projection of an outside corner of a building. The central axis is thus judged as though closer than the wings and therefore appears too small because of constancy scaling.

If Gregory's theory is correct and internally consistent, constancy scaling not only augments the apparent length of the central axis of Fig. 1a but also should augment its apparent width. That is, if constancy scaling is involved, both the apparent length and the apparent width of the central axis should be affected and should be distorted in the same direction.

The present study provides a direct test of the assumption of constancy scaling by determining whether subjects' length and width judgments of Müller-Lyer figures are distorted in the same direction. Because the Müller-Lyer figures usually consist of relatively fine lines, as shown in Figs. 1a and 1b, any illusion in the perception of the width of the central axis might not be noticed on casual observation. With thicker central axes, however, as shown in Figs. 1c and 1d, any illusion in the judged widths should be readily discernible. Note that these figures provide the same perspective depth cues as do the standard Müller-Lyer figures: the wings and their directions.

In the figures used here, the inward and outward wings met the central axis at 30 and 150 degrees, respectively. Four sizes of the central axis were used: 40 × 109, 27 × 136, 64 × 170 and 43 × 212 mm. Figures were presented one at a time, vertically as in Fig. 1, at a distance of 105 cm from the subjects and about 12 cm above eye level. The figures were projected on to a green blackboard with a slide projector.

Subjects were told to judge the apparent length and width of the central axis of each figure. The response sheet contained a horizontal and a vertical line which met and ended at a point near the upper left corner of the sheet. The subject responded by making a mark on each line to indicate the length and width of the central axis. The figure remained present while the subject made his response. No more than two subjects were tested at a time. Fourteen subjects judged each of the eight figures six times in randomized blocks of trials. Responses were measured to the nearest millimetre.

The results of the experiment contradict Gregory's theory. Fig. 2 shows that the Müller-Lyer illusion occurred:

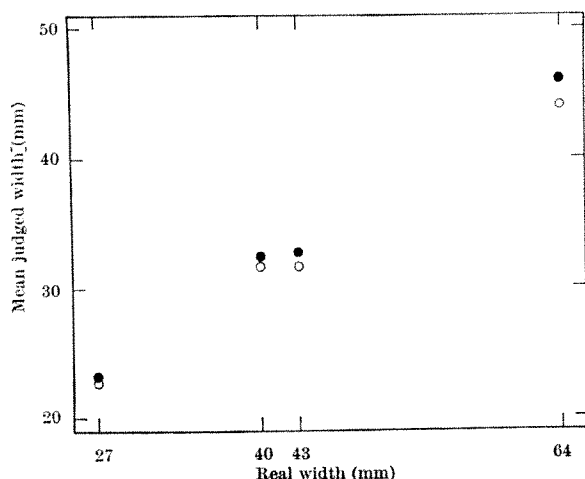


Fig. 3. Mean judged width of the central axes of Müller-Lyer figures as a function of the absolute width and the direction of the wings (●, inward; ○, outward).

the lengths of the central axes with outward-directed wings were judged longer than the corresponding axes with inward-directed wings. As explained above, it follows from the theory that the width judgments should have been distorted in the same direction; but they were not. Fig. 3 shows that the width judgments were, rather, distorted in the opposite direction: the widths of the central axes with outward-directed wings were judged smaller than the corresponding axes with inward-directed wings. A repeated-measures analysis of variance revealed that this average difference (1.13 mm) was significant ($P < 0.01$).

One of the most popular explanations of the perceptual distortion of the lengths of these figures has been Gregory's perspective theory. But this theory does not predict the judgments of width obtained here. This study was a critical test, for the figures used have substantial cues for depth and should, according to the theory, have triggered constancy scaling. Given its inability to account for the perceived width of the figures, Gregory's theory cannot provide a consistent account of the Müller-Lyer illusion.

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Facets of Control in Human Walking

Using eight normal barefoot male subjects, we have studied, for both feet simultaneously, the timing characteristics of the various phases of heel and toe contact with the ground. Two types of experiment were performed for walking on the level, one with speed fixed at 4.5 feet/s while pace frequencies were varied between 1.25 and 4.00 per s; another with speeds of natural walking ranging from 2.0 to 6.7 feet/s. We chose to experiment with barefoot subjects to eliminate the possible influence of different footwear, recognizing that walking characteristics, with and without footwear, would not necessarily be identical. For both natural and forced pace walking

we found that averages of the various footswitch phases, when normalized with respect to the average periodic time, remained fairly constant. Also, for natural walking, both pace frequency and step size seem to depend on the square root of the speed.

The barefoot subjects had shaped, fine mesh stainless steel electrodes taped over the heel and toe regions of both feet on top of an insulating layer of adhesive tape. The toe electrode covered the area under the big toe as well as the pad of the foot directly behind the toes, and the heel electrode covered the load bearing parts as indicated by the thick-skinned regions. They kept pace with a motorized cart whose speed could be controlled, and after traversing some 20 feet over which a steady-state walk could be reached the subjects walked on a roughened sheet of 1/16 inch aluminium 30 feet long and 18 inches wide.

A small junction box on a belt around the subject's waist held four resistors with values in the proportions 8 : 4 : 2 : 1 and commoned at one end. The other ends of each resistor were connected to their respective footswitch electrodes and the common junction was connected to a 6 V battery and a resistor in series mounted on the cart. The aluminium walkway was connected to the latter resistor by way of the shield of a signal cable running from the cart. The footswitch states determined the voltage appearing across the resistor. This voltage was led through the signal cable directly to our digital computer which was programmed to sample it at the rate of 200 Hz while the subject was over the aluminium surface. Then the programme translated the signal into graphic responses associated with each footswitch and effected analyses of the data acquired for the number of steps taken over the 30 foot length. Average values of the various phases of walking were computed, together with their mean deviations, variances and standard deviations. With the onset of the right-heel-down phase as the starting condition, two average down phases of each footswitch performance were plotted. Results were derived from the averages of three to twelve sampled steps depending upon speed of walking or frequency of pacing. The maximum mean deviation recorded was 0.078 and the maximum standard deviation 0.084 for a time period of around 0.5 s. In general, values for these deviations were considerably less. Various plots were made from the computed data.

Fig. 1 is typical and shows average periodic time as measured for the onset of the right heel down state plotted on the abscissa, with ordinates each expressed as a fraction of the average periodic time as follows: RHD, the time for which the right heel is down; RTD, the time

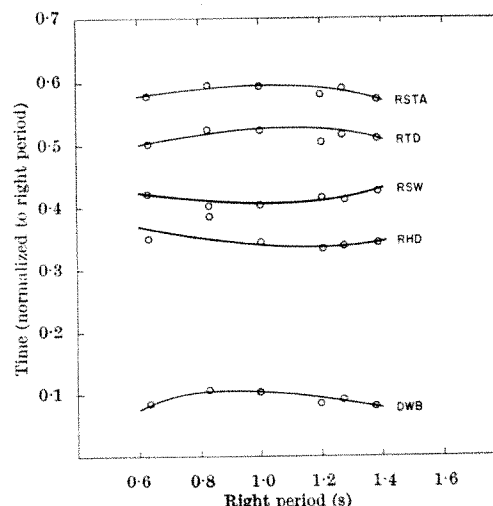


Fig. 1. Timing normalized to right period of footswitch phases against right period.

Table 1. PHYSICAL DATA OF SUBJECTS AND AVERAGES OF FOOTSWITCH PHASES FOR SPEED 4.5 FEET/S

Subject	Weight pounds	Height Feet Inches	RSTA	RSW	RTD	RHD	DWB
FH	175	5 10	0.59	0.41	0.51	0.34	0.1
MM	191	5 10.5	0.6	0.4	0.55	0.36	0.1
CM	195	5 11.25	0.61	0.39	0.55	0.33	0.11
EPE	196	5 11	0.60	0.40	0.52	0.37	0.1
FM	150	5 10	0.60	0.40	0.54	0.35	0.1
RL	214	6 0.25	0.63	0.37	0.56	0.44	0.13
RVB	158	5 10	0.58	0.42	0.55	0.30	0.08
AOQ	133	5 8	—	—	—	—	—

Each of the factors RSTA, RSW, RTD, RHD and DWB is expressed as proportions of the time from right heel contact to right heel contact. RSTA is the right stance phase; RSW the right swing phase; RTD right toe down; RHD right heel down; DWB double weight bearing, being the phase for which both the left and right extremities are simultaneously in contact with the ground.

Table 2. VALUES OF THE CONSTANTS k AND n , FROM THE RELATION $f = kv^n$ OF PACE FREQUENCY f AND SPEED v , DERIVED BY PLOTTING RESULTS FOR EACH SUBJECT ON LOG-LOG PAPER

Subject	k	n
FH	0.44	0.49
MM	0.39	0.56
CM	0.43	0.50
EPE	0.43	0.50
FM	0.36	0.63
RL	—	—
RVB	0.46	0.50
AOQ	0.44	0.50

for which the right toe is down; RSTA, the right stance phase; RSW, the right swing phase, DWB, double weight bearing—the time for which both the left and right extremities are simultaneously in contact with the ground. In most cases variations about and with respect to the mean values of each of these factors were less than 10 per cent. Table 1 shows physical data relevant to the subjects and the averages of the various factors. The similarities between these factors for the various subjects, and their relative constancy, independent of the periodic time, and hence pace length, are noteworthy. Typical of the responses of all the subjects tested, Fig. 2 depicts the variation of the same factors as discussed in connexion with Fig. 1 but with varying speeds. At the top of Fig. 2 is a plot of the variation of period with speed. The factors RHD and DWB seem to produce the most significant percentage changes over the range of speeds. In all cases declines in RSTA, RTD, RHD and DWB with speed, and an increase in RSW, were found.

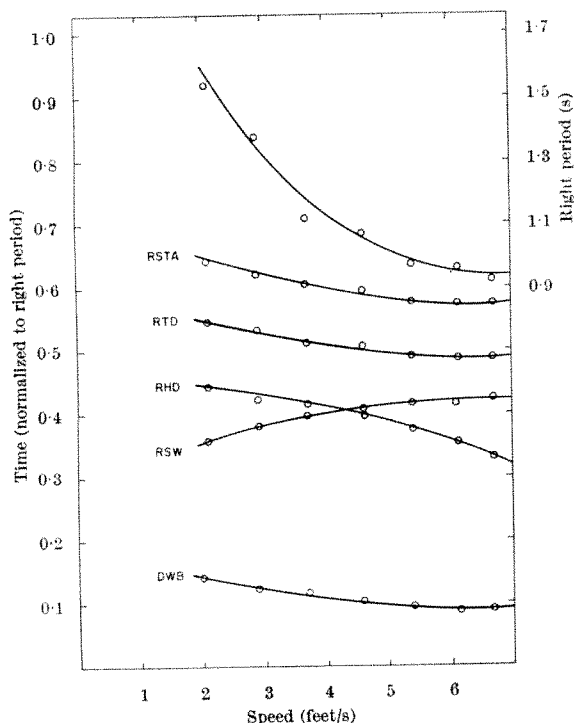
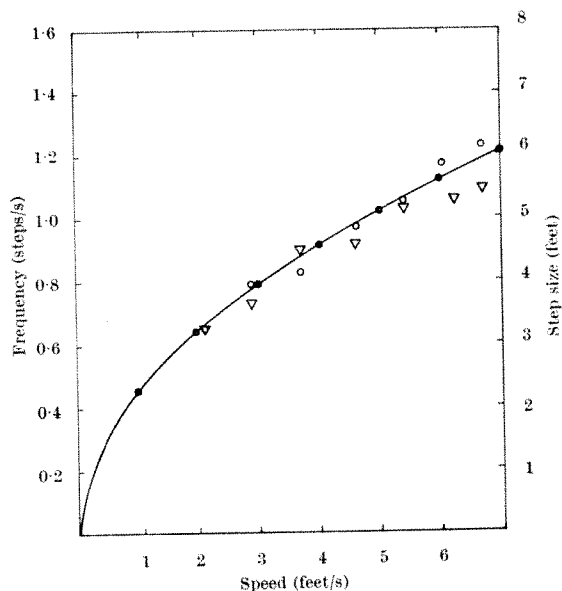


Fig. 2. Timing normalized to right period of footswitch phases for various speeds.

Fig. 3. Step size and step frequency against speed. ●, Fitted curve, $y = 2.28\sqrt{x}$; ○, step size; ▽, frequency.

The observation of the symmetrical properties associated with the left leg factors was interesting, though not unexpected. All the factors remain within a few per cent of their right leg counterparts.

Fig. 3 shows a plot of pace frequency f and the step size s derived from the relation $s = v/f$, against speed v . Also plotted is a curve whose equation is $y = 2.28\sqrt{x}$ and which seems to fit the step size data well. Plotting the results of the several subjects on log-log paper and assuming the relationship $f = kv^n$, where k and n are constants, their values turn out as in Table 2. It thus seems to a good approximation that pace frequency is dependent on the square root of speed and, as a consequence of the relation $s = v/f$, so is the step size. This shows an interesting aspect related to the control of walking speed: both pace frequency and step size increase with the square root of speed, and therefore pace frequency and step size are linearly connected. Control over the range of speeds examined is thus effected by the concomitant adjustment of these two parameters. Assuming, for the moment, that pace length alone decides speed, it is evident from geometric considerations for stiff-legged walking¹ that greater speeds will necessitate greater excursions in the centre of gravity of the body, thus calling for more lift energy per step. It would seem that the human locomotion system is so arranged as to cause increases in frequency of the pace in such a way as to compensate for increasing lift energy demands.

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Giraffid Horns

HENSHAW¹ has accepted Coope's premise² that giraffes have retained the skin-covered horn-like structures of the palaeomerycids, from which they are descended, because there have been no significant changes in the climate—the hypothesis being that the formation of antlers is a response to environmental temperatures. But the nature of the

giraffe horn can be seen in relation to the form of the animal and its social requirements, without any reference to the cervid antler³. As Henshaw states, the horn-like structures of the giraffe are analogous to the summer antlers of deer, in that they have points of resemblance, but there is no justification for invoking climate as an explanation of their form. They are specialized structures in the giraffe *Giraffa camelopardalis* L. of which the skin covering is no more velvet-like than that covering the rest of the animal's body, and it is certainly not vascular like the velvet skin of antler buds.

It is more pertinent to argue the case of the okapi *Okapia johnstoni* Selater in which the horns are primitive structures which have remained unchanged since the Miocene. But they have probably not developed further because elaborate structures would be a hindrance in the forest environment, and because the social behaviour of this solitary animal did not warrant the development of more than it already possessed.

The cervid antler must be shed and regrown from time to time because bone is brittle compared with horn and often becomes broken. An adult male giraffe, weighing some 1,500 kg, would require a massive palmate antler to withstand the weight of the body behind it if used in fighting. This, in its turn, would require much greater neck muscles, the volume increasing by the cube, and the whole weight of the giraffe would alter out of all proportion to the advantage gained, necessitating pillar-like legs to support it. We would, in fact, be left with a giant *Libythere*, an animal which has already evolved and faded out of the evolutionary picture.

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Directional Water-shedding Properties of Feathers

FEATHERS repel water strongly because they are porous. Their porosity can cause a large air-water interface, relative to solid-water interface, to form under a drop resting on the system of interlocked barbs (the vane). In this way the apparent contact angle (θ_A) increases to values far greater than any (θ) known for plane surfaces^{1,2}.

While investigating those structural properties of feathers which are responsible for their water repellency, I have found that drops of water on feathers tend to roll off distally. This is a clear adaptation to shedding water; a tendency for drops to roll proximally might cause wetting of the skin. The directional movement of water was studied by measuring the angle of tilt required to roll drops of water of known volume off the outer surface of freshly plucked breast feathers of several species, in eight directions of movement of the drop relative to the lie of the barbs (Fig. 1). Angles required for movement towards the base of the vane (directions B, D, X) were considerably greater than for movement towards the apex. A correlation was found between the tilt angle and the angle of movement of the drop relative to the distal barbules (Fig. 2). These project distally roughly parallel to the rachis, and overlies the proximal barbules and, to a varying extent, the barbs. To produce drop movement by tilting in the same directions on the ventral surface of the feathers, larger drop volumes were required. Here, tilt angles correlated with the angle made by the passage

of the drop relative to the lie of the barbs (Fig. 3); again, less tilting was required to roll drops distally than to roll them proximally, but tilt angles were greater than on the dorsal surface. In a particular direction of movement, both on the dorsal and ventral surfaces, tilt angle varied with drop volume (Fig. 4). A maximum drop volume was reached beyond which the same degree of tilting was required to cause rolling. Below a certain volume, the drop would not move. Tilting facilitates movement of the drop by increasing its gravitational potential energy, which acts against the tendency of the solid to attract the liquid. The variation of tilt angle with drop volume reflects this effect. A drop on a surface to which water is continuously added will reach a maximum height related to contact angle; if further water is added, the drop spreads³. Increasing drop volume on a feather beyond this point causes local spreading of the drop edge so that it makes new contact with asperities in the surface, which impede movement.

For a drop front to climb up and over a ridge, energy is required, and apparent advancing contact angles result which are larger than that on the flat surface: this is the so-called "edge effect"^{4,5}. It is interesting that directional water repellency similar to that on the vane was found on the non-coherent edge to the vane of breast feathers of *Alca torda*. Here, the barbules are filamentous and the distals project towards the tip of the feather at an angle of about 45° to the plane of the feather. Drops of water studied by a microprojector on this surface made contact with these barbules in different ways, according to whether the specimen was tilted downwards towards the tip or the base of the feather. In the former case, the advancing front of the drop overlaid the distals. In the latter case, the distals rose up at the drop edge to meet it; the mechanism for directional water repellency here

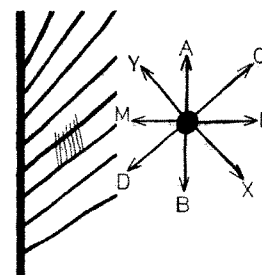


Fig. 1. Coherent vane of breast feather, showing barbs and distal barbules. Directions studied: tilting to A, distally, parallel to rachis; B, proximally, parallel to rachis; C, distally, parallel to barbs; D, proximally, parallel to barbs; E, distally, at 90° to barbs; F, proximally, at 90° to barbs; G, distally, at 90° to rachis; H, at 90° to rachis, towards rachis.

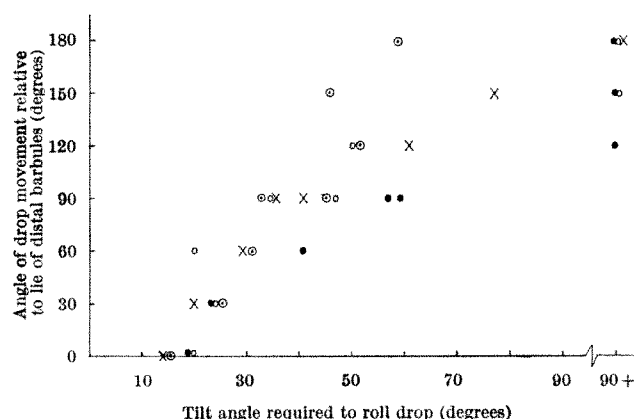


Fig. 2. Outer surface of breast feathers of \circ , *Larus marinus*; \circ , *Cygnus columbianus*; \bullet , *Anser canalicus*; \times , *Aythya australis*. Each point is mean of ten. Standard errors varied from 0.50 to 3.17 degrees of tilt. Drop volume 10 μ l. Tilt angles measured to nearest 5°.

is probably related to the ease of passage of the drop distally, by virtue of this effect, and the rising up of the distal barbules when the feather is tilted down basally impeded the travel of the drop. A similar effect was observed on breast feathers of *Podiceps cristatus* (museum skin). For the drop to move basally, the "ridges" are greater, and more gravitational potential energy is required for movement. That this explanation may also be applied to the vane of breast feathers is indicated by the finding that in *Phasianus colchicus*, where the distal barbules are relatively short and do not overlie the barbs (unlike the other species studied), little directional water repellency was found (Fig. 5).

It is thus likely that directional repellency is conferred in this way: as a drop moves distally off the feather, it presses down the distal barbules in its path. As it moves proximally, the barbules rise up against it. This mechanism must be considerably enhanced by the great degree of longitudinal curvature of breast feathers of water birds (work in progress). On the ventral surface, the barbs are presumably the operative ridges and their structure must be such that it is easier for a drop front to pass distally than proximally, that is, the solid angle⁴ in cross-section must be larger on the edge of the barb towards the feather tip than on the opposite edge. In the case of *Aythya australis*, microscopic examination of the inner surface of the feather shows that the tips of the proximal barbules lie along the barbs and project towards their tips. These barbule tips are raised above the plane of the barbs in ventral view and must therefore contact the water drop: for this reason it is possible that an effect similar to that described for the distal barbules occurs

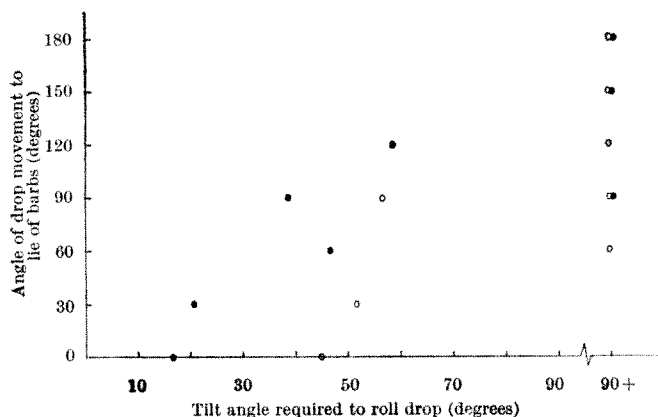


Fig. 3. Ventral surface of breast feathers of *Aythya australis*. Each point is mean of ten. Standard errors varied from 1.25 to 2.47. Drop volume 10 µl (○), 20 µl (●). Tilt angles measured to nearest 5°.

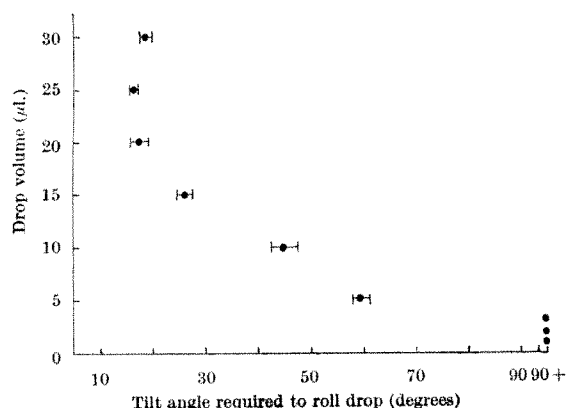


Fig. 4. Inner surface of breast feathers of *Aythya australis*; drop movement in direction A. Each point is mean of ten. Standard errors indicated. Tilt angles measured to nearest 5°.

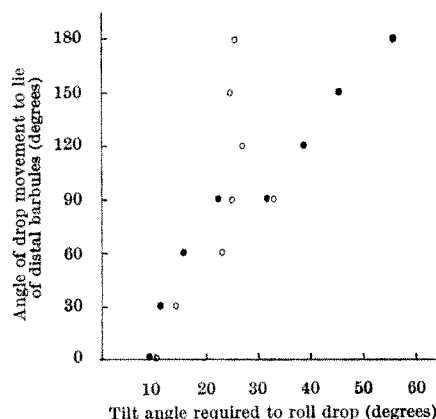


Fig. 5. Outer surface of breast feathers of *Phasianus colchicus* (○) and *Corvus monedula* (●). Drop volume 10 µl. Each point is mean of ten. Standard errors varied from 0.50 to 4.40. Tilt angles measured to nearest 5°.

here also. It should be noted that movement of water drops in this way requires a large receding contact angle, and configuration of asperities such that no water is left behind⁴. Further work on this feature of feathers is in progress.

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DDE thins Eggshells of Captive American Kestrels

EGGSHELL thinning in several species of raptorial and fish-eating birds, whose populations and/or reproductive success have declined dramatically in recent years, has been correlated with residues of DDE [1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene] in their eggs¹⁻³. DDE, a common metabolite of DDT [1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] and a nearly universal contaminant in the food of these birds, has been considered to be the major chemical responsible for eggshell thinning.

Heath, Spann and Kreitzer induced significant eggshell thinning and cracking in penned mallards (*Anas platyrhynchos*) by feeding the birds low dietary levels of DDE⁴. To determine if this chemical would also cause thinning of the eggshells of the American kestrel (*Falco sparverius*), we placed twelve pairs of hawks on a diet containing 10 p.p.m. p,p'-DDE on a dry weight basis (2.8 p.p.m. on a wet weight basis), beginning on March 30, 1968. This dosage was equivalent to residue levels commonly found in food items of raptors in the field⁵⁻⁸. The DDE (99 per cent pure) was dissolved in cottonseed oil and added to a diet of ground meat. The hawks were continued on the dosage until the experiment ended in 1969. No insecticide was added to the diet of ten pairs of non-dosed hawks. Clean oil had been added to their food since March 11, 1966, in amounts equal to that added to the diet of the dosed group. Both groups of hawks, dosed and non-dosed, were similar in age, but had somewhat different histories.

In 1968 and 1969, we collected the first egg laid by all pairs in both groups, where possible, for determination of shell thickness and pesticide content. Methods for handling shells and contents of the eggs are given elsewhere⁹.

Table 1. DDE RESIDUES AND SHELL THICKNESS OF KESTREL EGGS

Diet and year	Eggs analysed for residues	Av. residues (p.p.m. wet weight) \pm s.e.*	Range	Shells measured	Average thickness (mm) \pm s.e.	Range	Percentage thickness decline (1969)
Non-dosed							
1968	10	0.62 \pm 0.05	0.37-0.80	10	0.188 \pm 0.007	0.130-0.210	
1969	10	0.71 \pm 0.05	0.38-0.96	10	0.184 \pm 0.008	0.130-0.210	2.1
DDE dosed							
1968†	10	3.09 \pm 0.45	1.05-5.89	8	0.186 \pm 0.005	0.165-0.203	
1969	9	32.4 \pm 2.78	17.4-44.2	8	0.168 \pm 0.004	0.153-0.185	9.7‡

* Lipid weights averaged 6.5 per cent of the fresh weight of egg contents.

† In two cases where females laid their first egg before initiation of dosage, residues from a later egg in their first clutch were reported.

‡ Significant decline ($P < 0.001$), paired t -test. Only eggs from females that laid both years were compared.

In 1968, the hawks in the dosed group laid their eggs from one day before dosage began (two pairs), to seven weeks after dosage began; in 1969 these hawks had been on dosage approximately one year when they laid their eggs.

Eggs were analysed for chlorinated hydrocarbons by the WARF Institute. Samples of the entire egg contents were oven dried at 40° C for 24-36 h, ground with sodium sulphate and extracted with a mixture of ethyl ether and petroleum ether (70 : 170) for 8 h in a Soxhlet. Clean-up and preliminary separation were by two elutions through a florisil column (5 per cent ethyl ether in petroleum ether and 15 per cent ethyl ether in petroleum ether). Analysis was by electron capture gas chromatography (Barber Coleman Pesticide Analyser model 5360, Sr 90). The first elution was analysed on a 5 per cent DC 200 on Cromport XXX at column temperature 195° C; the second and check samples of the first were analysed on a 5 per cent QF-1 on Cromport XXX at column temperature 180° C. Injector temperatures were 230° C; detector temperatures were 240° C; nitrogen flow rates were 70-90 ml. per min. Columns were glass, 4 feet \times 4 mm. Polychlorinated biphenyls (PCB) were identified by peak matching and confirmed by dehydrochlorination with KOH, but were not quantified.

Average shell thickness of eggs laid by our hawks in 1968 is compared in Table 1 with thickness of eggs laid by the same females in 1969. Average shell thickness of eggs laid by DDE-dosed hawks in 1969 was 10 per cent less than in 1968 ($P < 0.001$); the 2 per cent decrease in average shell thickness of eggs laid by non-dosed hawks in 1969 was not statistically significant.

Earlier, we induced eggshell thinning in kestrels by feeding them a diet containing DDT in combination with dieldrin (composed of 95 per cent HEOD which is 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene)⁹. Because DDT metabolizes to DDE, the DDE stored in the birds' tissues probably contributed to the thinning of these eggshells. Dieldrin has been shown to cause eggshell thinning in mallard ducks¹⁰, but not in pheasants (*Phasianus colchicus*)¹¹. Its contribution to eggshell thinning in combination with DDT and/or its metabolites is not known.

The DDE residues found in the eggs are reported in Table 1. All are based on the fresh weight of the egg contents. The eggs from the non-dosed group contained an average of less than 1 p.p.m. DDE during both years; those of the dosed group contained an average of 3 p.p.m. DDE in 1968 and an average of more than 30 p.p.m. in 1969. Average residues of DDT plus DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] did not exceed 0.28 p.p.m. in any experimental group during either year. No group contained an average of more than 0.06 p.p.m. dieldrin. PCB occurred in comparably small amounts.

Judging from the levels of DDE found in our kestrel eggs in 1969, the amounts of DDE reported from twenty-seven British peregrine falcon (*Falco peregrinus*) eggs (av. 15.7 p.p.m.; range 2.6-30.8 p.p.m.)¹² may have been large enough to have caused some of the eggshell thinning reported for that population¹³. High levels of DDE have also been reported in seven peregrine eggs from Northern Canada (range 10.4-41.8 p.p.m.)¹⁴; eggshell thinning in this population has not been documented.

DDE-induced thinning of kestrel eggshells in a controlled experiment further strengthens the hypothesis that this chemical is an important factor in eggshell thinning in wild raptorial and fish-eating birds.

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Acid and Alkaline Phosphatase Activity in the Liver of Brown and Rainbow Trout

THE susceptibility of the rainbow trout to liver cell tumours following the administration of aflatoxin is well documented¹⁻³, as is the refractory behaviour of brown trout⁴. The different responses of these two closely related species probably indicate variations in basic metabolism. We decided to investigate histochemically some enzyme systems of the livers from brown and rainbow trout. Although histologically these livers were similar, the activity and distribution of the acid and alkaline phosphatases showed distinct variations. The activity of alkaline phosphatase was greater in the brown than in the rainbow trout (Fig. 1a and b), while acid phosphatase was more active in the rainbow than in the brown trout (Fig. 1c and d). The distribution of the phosphatases varies throughout the fish kingdom^{5,6}, and the significance of the present findings is uncertain. The metabolic differences in these two species may begin to explain the inverse response to the induction of liver cell tumours. A significant loss of alkaline phosphatase from the livers of ducklings fed aflatoxin has already been reported⁷, as has the elevation of various serum enzyme concentrations, including alkaline phosphate, in the pig⁸. We are extending histochemical studies on the effect of aflatoxin at various doses on the two species of trout, and

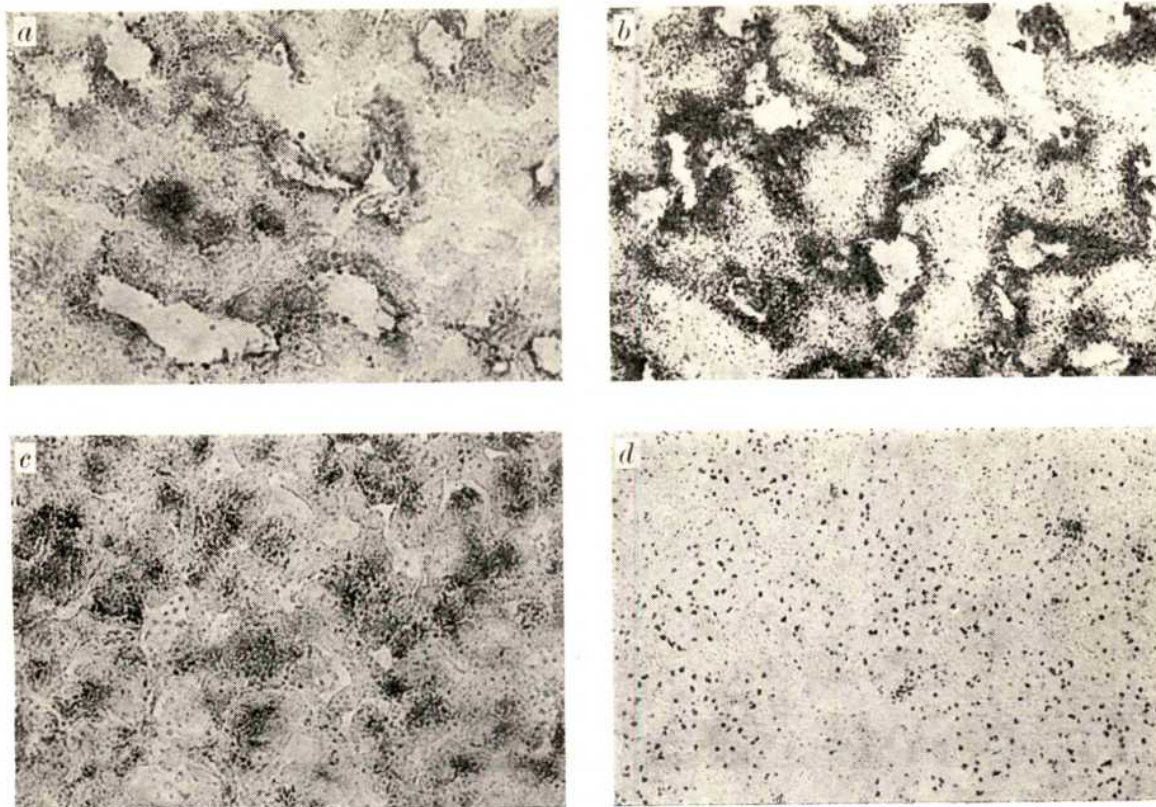


Fig. 1. a, Rainbow trout, alkaline phosphatase in liver; b, brown trout, alkaline phosphatase in liver; c, rainbow trout, acid phosphatase in liver; d, brown trout, acid phosphatase in liver.

hope to elucidate the different effects, if any, on the enzyme systems in the livers with special reference to the phosphatases.

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Evidence for *Anopheles squamosus* Migration?

IN normal tropical and sub-tropical conditions most species of anopheline mosquitoes usually fly only short distances in search of food, shelter and breeding sites. Experimental investigations on *Anopheles funestus*¹, *A. gambiae*² and *A. melas*³ in Africa, *A. quadrimaculatus*⁴ in the United States of America, *A. argyritarsis*⁵ in Canada, *A. flavirostris*⁶ in the Philippines and *A. culicifacies*⁷ in India, have demonstrated that most adult mosquitoes

do not normally fly more than 3–5 km from a site of origin or release.

Although it is very difficult to prove that mosquitoes can disperse far afield, one African anopheline, *Anopheles pharoensis*, has been credited with performing migratory flights over considerable distances⁸. This species has been recorded on separate occasions as having apparently travelled over 29 km⁹, 56 km¹⁰ and perhaps as much as 280 km⁸ in the deserts of North Africa and the Middle East. Several such migrations have been correlated with the occurrence of a full moon⁸.

Another species, *Anopheles argenteolobatus*, belonging to the same subgenus and series as *A. pharoensis* (*Cellia*, *cellia*), has been reported to show remarkable fluctuations in density at the edge of its range in Southern Africa^{11,12}, with the possible interpretation that this species too may sometimes migrate to new territory. A further species, *A. squamosus*, similarly belongs to the same subgenus and series as *A. pharoensis*, the larvae being virtually indistinguishable. I report here that numbers of *A. squamosus* were recently collected at Amani in Tanzania under circumstances suggesting the arrival of an immigrant swarm.

Amani is a diffuse human settlement, situated at an altitude of about 1,000 m atop the heavily forested Eastern Usambara Mountains of north-eastern Tanzania. Since 1951, the East African Institute of Malaria and Vector-Borne Diseases has been located at Amani. Staff of the institute have carried out much malariological work in the district, including detailed mosquito surveys, with one result being that during the years from 1951 to 1967 *A. squamosus* was not recorded at Amani. The species is known to be relatively common, however, in some parts of the Usambara foothills, below the forested level, at altitudes around 200 m and distances of at least 10 km from the institute at Amani¹³.

For several years a mercury vapour lamp has been operated irregularly in the institute's grounds for collecting

insects. On the evening of December 23, 1968, the lamp was switched on at dusk (1830 h), after having not been used for some weeks previously. When visited at 2100 h a number of *Anopheles squamosus* females were observed among the insects attracted to the lamp. By 2200 h a total of thirty-one *A. squamosus* females were collected, but no more arrived thereafter. Twenty specimens were dissected later, of which fifteen were found to be unfed and with resting-stage ovaries, while five were fully gravid. Two of those with undeveloped ovaries were parous and the remaining thirteen nulliparous. All females contained sperms in the spermatheca. No malaria or filaria parasites were found.

Meteorological conditions at Amani were steady and typical for the season during the week leading up to December 23. A short rainy season had occurred throughout the region during November, with rainfall for the month totalling more than 15 inches at Amani. Rainfall on December 23 and for the previous five days was nil. On December 24 the maximum temperature was 27.3° C, the minimum temperature 16.1° C and the relative humidity was 60 at 1500 h and 86 at 0900 h. A new Moon had first appeared on the night of December 21, but on the 23rd there was complete cloud cover in the evening and slight mist.

Table 1. NUMBERS OF *Anopheles squamosus* COLLECTED AT LIGHT NIGHTLY BETWEEN DUSK (1830 H) AND 2200 H AT AMANI, TANZANIA

Date	<i>A. squamosus</i>	
	Males	Females
23rd December, 1968	0	31
24th " "	0	6
25th " "	0	2
26th " "	1	22
27th " "	0	4
28th " "	—	—
29th " "	1	17
30th " "	0	14
31st " "	1	8
1st January, 1969	0	1
2nd " "	0	0
3rd " "	—	—
4th " "	—	—
5th " "	0	1
6th " "	0	0
7th " "	0	1
8th " "	0	0
9th " "	0	1
10th " "	—	—
11th " "	0	0
12th " "	0	0
13th " "	0	3
Totals	3	111

* Collection at dawn.
† No collection.

On ensuing nights more *A. squamosus* were attracted to the lamp soon after dusk. The numbers collected each evening by 2200 h are shown in Table 1. Moderate catches, including three males, were made during the last week of December and a few more specimens were caught in the first half of January. Very few specimens arrived later than 2200 h and, although the lamp was always left on until after dawn, daily inspections at 0730 h yielded a total of only three additional *A. squamosus* females. The only other anophelines collected during the entire period were single females of *A. ziemanni* and *A. coustani*.

All possible anopheline breeding places within a mile of the lamp were searched on December 24, 27 and 30, 1968, January 2, 7 and thereafter at approximately weekly intervals until the end of March 1969. No *A. squamosus* larvae were discovered and there has been no other indication that the species managed to establish breeding in the area. The lamp failed to attract any more *A. squamosus* when used on numerous occasions during 1969 and on every night during December 1969 and January 1970.

In order to reach Amani by flying, *A. squamosus* adults would have needed to indulge in appetential behaviour¹⁴ of a migratory nature quite distinct from the usual short-range journeys in search of food, resting places and so forth. Even if passively wind-borne, in similar fashion to the migration of frit-flies and aphids¹⁵, the resulting dispersal of *A. squamosus* apparently contrasted with the

trivial flights regularly performed by mosquitoes in general and resembled the special migratory behaviour known to be a feature of *A. pharoensis*.

The failure to detect more *A. squamosus* at Amani during the 14 months following these observations supports the belief that this immigration was an unusual and isolated event. There is no way of determining the place of origin of the mosquitoes, except that it was probably at least 10 km away from Amani in the mountain foothills or beyond. Because the catch included some males it is feasible that the specimens collected might have bred locally. The sudden and transient incidence of *A. squamosus* at Amani can, however, only be accounted for by immigration of a population which did not survive.

In tropical situations direct evidence for the dispersive movements of wild mosquitoes is difficult to acquire. These observations on *A. squamosus* indicate that this species may resemble *A. pharoensis* and possibly *A. argenteolobatus* in performing flights of a migratory nature,

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Age Dependency of Response in an Insect Chemoreceptor Sensillum

THE electrical responses of contact chemoreceptors on the mouthparts of many species of Diptera can determine whether or not a fluid medium is ingested. In the blowfly *Phormia terranova*, the responses of the large bristle-like sensilla, which occur in two aboral patches on the labellar lobes, regulate the extension of the proboscis and the opening of the labellar lobes. The responses of a single sensillum may mediate this action if stimulated with water or with certain sugars in aqueous solution¹. Without extension and opening, feeding is impossible. Rejection thresholds seem to be determined to some extent by the responses of the type 1 ("salt") receptor cell, one of the four types of chemoreceptor cell contained within each sensillum².

I summarize here some evidence of changes in sensitivity of two of the receptor cell types, 1 and 3 ("water"), which seem to be correlated with the age of the adult insect and which suggests that insect age may need to be considered in a full analysis of feeding behaviour in flies. The electrical responses of types 1 and 3 receptor cells which result from stimulation with water and various electrolyte solutions are now well known^{3,4,7,8} and represent preparations made from adult flies aged between 3 and 5 days from emergence from their puparia. Using flies maintained at 25° C in conditions of alternate periods of 12 h darkness and 12 h illumination (about 450 foot candles),

and provided with sucrose solution and raw meat as food, the electrical responses of cell types 1 and 3 were measured in flies of known ages up to 30 days, in all cases during the lighted phases of the illumination cycles. The stimulus solutions were water and NaCl (0.1, 0.2, 0.5 and 1.0 M). Ten flies were sampled on each successive day and the responses of four of the longest sensilla in each labellar preparation were recorded. (These sensilla may be defined from Wilczek's map⁸ as "largest, right 10, 11 and left 10, 11"; they are about 350 μ m in projecting length.) Each was stimulated four times with each of the test solutions, applied in a random sequence.

The responses are plotted as the mean adapted frequency of action potential generation against age for type 1 cells (Fig. 1a) and for type 3 cells (Fig. 1b). Any cells which failed to respond were ignored in calculating the mean discharge frequencies. In this connexion there was an increasing incidence of such cells among the samples of receptors, in which the ability to generate impulses had been altogether lost as the age of the flies increased. In Fig. 2, the incidence of type 1 cell failure (no response to 1.0 M NaCl) as a percentage of the forty cells in each daily sample has been plotted against age. It was rare to find an insect where response failure had affected some rather than all or none of the four cells examined. Between days 16 and 25, the mean incidence of type 1 cell failure in all the flies remains approximately constant near 60 per cent, but when flies were analysed individually only 27 per cent of the total sample of 100 insects between these age limits showed partial receptor failure (Fig. 3). This indicates

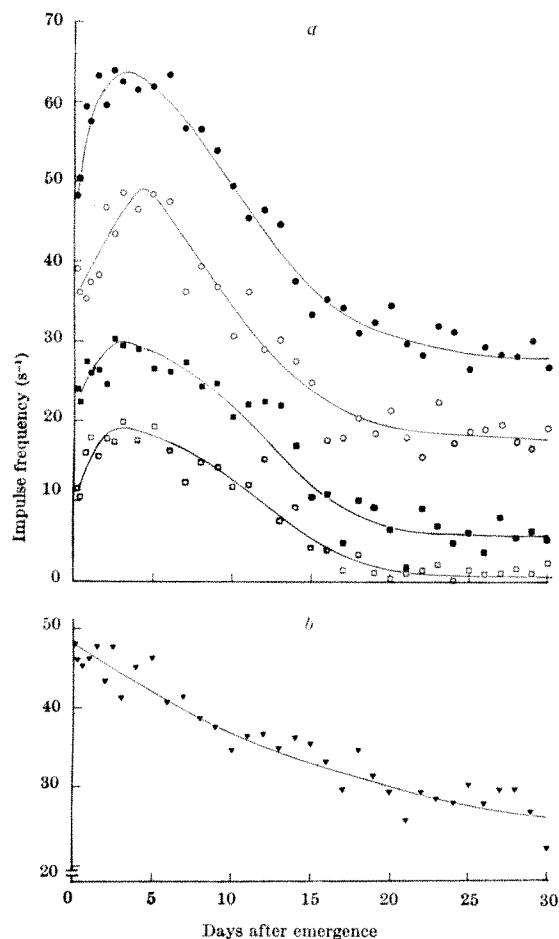


Fig. 1. *a*, Mean action potential discharge frequencies of type 1 receptor cells (after 350 ms of stimulation) as a function of insect age. Between days 4 and 30 (inclusive) each daily age sample was obtained over a period $\ll 8$ h. Stimulus solutions: \bullet , 1.0 M NaCl; \circ , 0.5 M NaCl; \blacksquare , 0.2 M NaCl; \square , 0.1 M NaCl. The responses of ten receptors represent the sample for each day, except on day 1 (30) and days 2 and 3 (20). *b*, As *a*, but for responses of the type 3 receptor cells stimulated with distilled water.

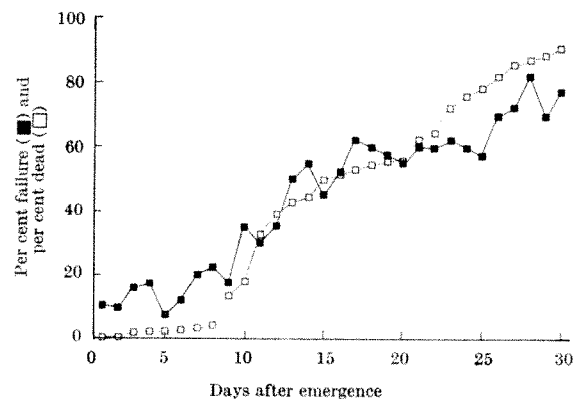


Fig. 2. The dependence of the percentage of inoperative chemoreceptor cells (type 1) (\blacksquare) upon the age of the insect. Sample sizes: day 1 (120 cells), days 2 and 3 (80 cells), all others (40 cells). The diagram also shows the mortality (\square) of flies in a parallel culture of 500 insects kept in the same conditions as those used in the experiments.

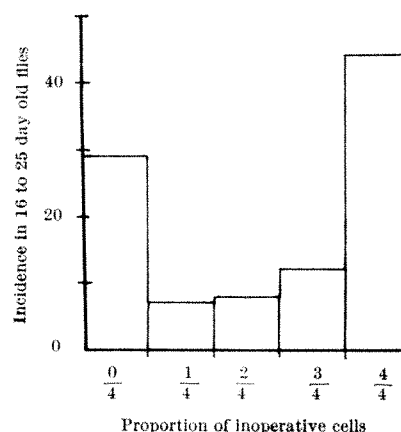


Fig. 3. Incidence of differing extent of chemoreceptor (type 1) failure per fly, in a sample of 100 flies of mixed ages between 16 and 25 days inclusive. Four cells in each fly were examined.

that there is some synchronization of failure in an individual fly. Occasionally, cells were found where the receptor potential could still be elicited, but where impulse generation was disabled.

In type 1 cells, the electrical responses are maximal when stimulated with NaCl solutions at about day 3. The response threshold rises with age after 3 days, but after day 20, the decline in sensitivity is more gradual. In type 3 cells, however, the discharge frequency diminishes steadily from the day of emergence of the fly. The rejection of a solution for ingestion can be brought about by stimulation of the type 1 cell, even in the presence of sucrose which, applied alone, elicits acceptance through stimulation of the type 2 "sugar" receptor, and therefore the consequence of a decline in sensitivity of the type 1 cell might be to allow acceptance of a wider range of solutions as food. If flies become less well able to forage for food as they age, it is conceivable that the survival value of an ability to ingest materials more often encountered, even if of less suitable content, may exceed that where only a more ideal food can elicit the ingestion response.

The type 1 cell discharge frequency increases with concentration of applied sodium chloride, whereas that of the type 3 cell decreases. Cohen has suggested⁶ that two such "opposing" receptors may be used to detect a stimulus intensity, here concentration, which elicits a combined minimum or zero output from both. In 3 day old flies, when the threshold of the type 1 receptor is at its lowest level, there is only a restricted concentration zone, between 60 M and 70 mM NaCl, which does not evoke a response from either type 1 or 3 cells. With increasing age, this silent zone widens; at 20 days it extends from

25 mM to 95 mM NaCl, and the properties for precise measurement of concentration of the opposing receptor system that are potentially present in younger insects are lost.

It is interesting that the mortality plot for these flies (superimposed on Fig. 2) shows a similar time-course to the plot of incidence of receptor cell failure. It is difficult to understand how a fly could feed at all if there were no peripheral chemosensory drive to initiate the reflex opening of the labellar lobes or the eversion of the proboscis. Perhaps massive chemoreceptor failure may be a factor of which the time of onset is important in determining the life span of the fly, especially because there is some evidence (Fig. 3) that such failure is approximately synchronized in any individual fly. Other sensilla on the tarsi of these flies may, however, initiate the proboscis extension reflex¹, and other chemoreceptor cells within the labellar chemoreceptors exist for which age dependent changes in response have not yet been investigated, notably the type 2 or "sugar" receptor, a cell of great importance in initiating the eversion and opening reflex.

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Magnesium as a Toxic Element

THE soils over serpentine rocks are renowned for their barrenness, the causes of which vary enormously because the term "serpentine" embraces a wide variety of soil types. Sometimes, for a variety of reasons, these soils are very toxic to certain plants.

Hunter and Vergnano¹ observed toxicity symptoms in oat plants growing in a soil contaminated with serpentine. They reproduced these symptoms in culture by adding nickel to the media and correlated the symptoms with the content of nickel in the plants. Soane and Saunder² presented data which suggested that chromium, as well as nickel, toxicity might be important in some Rhodesian serpentine soils.

One Scottish serpentine soil, from near the hill known as Meikle Kilrannoch, in Glen Doll, Angus, is very toxic in greenhouse conditions. *Avena sativa* (oats variety 'Victory') and a non-serpentine race of *Agrostis stolonifera*, collected from Oxford, are very stunted when grown in this soil, with root growth almost completely inhibited. I have investigated the causes of this toxicity.

Soil for analysis (Table 1) was collected from the top 6 inches of the barest areas of the site, sieved (1 mm pores for chromium and nickel, otherwise 2 mm), and air dried. Normal ammonium acetate was used to extract the exchangeable cations, which were analysed by atomic absorption spectrophotometry. Phosphorus was extracted by Truog's reagent—0.002 N sulphuric acid plus 3 g/l. of ammonium sulphate; it was determined colorimetrically.

Table 1. ANALYSIS OF TWO SOILS FROM MEIKLE KILRANNOCH

	Exchangeable cations (mequiv./100 g)			Phosphorus soluble in dilute acid p.p.m.	Total Ni and Cr (p.p.m., semi-quantitative)		pH (1 : 2.5 soil-water suspension)
	Mg	Ca	K		Ni	Cr	
(a)	11.9	0.3	0.05	2	1,000	4,000	6.8
(b)	10.1	0.5	0.04	2	1,500	4,000	6.7

Nickel and chromium were analysed by X-ray fluorescence, and pH was determined in a 1 : 2.5 soil-water mixture. All the above ground parts of the oats grown in the Meikle Kilrannoch soil were used for analysis (Table 2) after digestion of dried samples in concentrated nitric acid. Calcium and magnesium were determined in an EEL atomic absorption spectrophotometer, potassium in an EEL flame photometer and nickel and chromium by X-ray fluorescence in a Philips Pw 1540 spectrograph. A reference analysis of healthy oats grown on a fertile soil is included in Table 2.

Table 2. METAL CONTENT OF OAT SHOOTS

	Ca	Mg	Ni	Cr
Meikle Kilrannoch soil	1,262	11,020	20	< 10
John Innes potting compost No. 2	4,735	2,330	< 5	< 10

Figures are p.p.m. of material oven dried at 105°C.

The soil had high contents of nickel and chromium, but they are unlikely to have been responsible for the toxicity. The symptoms shown by the plants grown on this soil were not those of heavy metal toxicity, and moreover there was comparatively little heavy metal in the plants. These plants contained much less nickel and chromium than those growing in another, non-toxic, serpentine soil³.

Tissue of *Agrostis canina* growing at Meikle Kilrannoch was analysed with a quartz glass Hilger spectrograph of the Littrow type for Ni, Cu, Mn, Co, Cr, Fe, Al, Mo, Zn, Pb, Ba and Ti, but none of these seemed likely to be present in quantities large enough to be toxic.

The possibility that magnesium ions were toxic was assessed in water culture experiments. The influence of magnesium ions on (a) a serpentine clone of *Agrostis canina* from Meikle Kilrannoch (clone MK/1) and (b) a non-serpentine clone of *A. stolonifera* from Oxford (clone PC/2) was investigated using single salt solutions of magnesium sulphate. Six concentrations of magnesium were used, and every treatment had eight replicate jam jars of about 330 ml. capacity, covered with aluminium foil, each containing two rootingtillers from each clone. The growth of the roots was measured first after 48 h in distilled water, after which the roots were transferred to the test solutions and measured after a further 48 h. Results are expressed as the ratio of root grown in test solution to root grown in distilled water (see Table 3). Ratios were satisfactorily distributed and not transformed before analysis.

Clearly magnesium ions can be surprisingly toxic in single salt solutions. (No difference was found between the toxicities of magnesium sulphate, chloride and nitrate³.) Moreover, the serpentine clone was tolerant of higher concentrations of magnesium than was the non-serpentine clone.

The difference in ability to grow in a magnesium solution is not fortuitously connected with the specific difference between *Agrostis stolonifera* and *A. canina*, for magnesium susceptible races of *A. canina* and tolerant races of *A. stolonifera* have been found at other sites³.

Magnesium toxicity in both soil and water culture can be ameliorated by the addition of calcium, and magnesium is toxic only when the concentration of calcium is sufficiently low. For example, 0.5 p.p.m. of Ca⁺⁺ added to a solution containing 10 p.p.m. of Mg is sufficient to cause a substantial, if not complete, amelioration of toxicity.

Table 3. MEAN RATIO OF LENGTH OF LONGEST ADVENTITIOUS ROOTS IN TEST SOLUTION TO LENGTH OF LONGEST ADVENTITIOUS ROOTS IN DISTILLED WATER OF CLONE PC/2 AND MK/1 ± STANDARD ERROR OF MEAN

Clone	Magnesium (p.p.m.)					
	0	0.5	1.0	2.5	5.0	10.0
MK/1	1.03 ± 0.069	0.94 ± 0.059	0.90 ± 0.084	1.14 ± 0.056	1.00 ± 0.084	0.97 ± 0.089
PC/2	0.82 ± 0.099	0.92 ± 0.056	0.75 ± 0.059	0.56 ± 0.043	0.27 ± 0.016	0.15 ± 0.027

Although the soil from Meikle Kilrannoch probably represents an extreme in toxicity, adverse effects of high concentrations of magnesium in the presence of low soil calcium are almost certainly of widespread importance on serpentines. Walker⁴ and Kruckeberg⁵ observed reduced growth of non-serpentine plants in some American serpentine soils. They hinted at possible toxic effects of magnesium, but concluded that calcium deficiency is the principal cause of the reduced growth in the serpentine soil. This interpretation has practical value because the deleterious effects on plant growth can be cured by the addition of calcium. But the emphasis on a calcium deficiency rather than a magnesium toxicity is unfortunate because (a) non-serpentine soils with comparably low contents of calcium do not affect plant growth in a manner similar to serpentine soils, and (b) plants can be actively poisoned by the toxic soils and do not show symptoms of deficiency. It seems that calcium is deficient only in the context of high concentrations of magnesium. Recent work on calcium⁶ has suggested that a major function of this element is to ameliorate the toxicities of other elements.

This work throws light on another interesting problem. A non-serpentine maritime race of *Armeria maritima* produced much healthier growth on the Meikle Kilrannoch soil than would have been expected from the results for oats and *Agrostis stolonifera*. Kruckeberg⁵ found that maritime species tolerated serpentine soils, but he had no explanation for this. In view of the high ratio of magnesium to calcium in seawater, perhaps maritime plants are to some extent preadapted to serpentine soils. Magnesium toxicity might be an important and hitherto under-considered factor in the maritime environment.

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Resonance Raman Scattering of Laser Radiation by Vibrational Modes of Carotenoid Pigment Molecules in Intact Plant Tissues

We report here the detection of resonance Raman (RR)¹⁻¹¹ scattering of laser radiation from vibrational modes in the molecules of the carotenoid pigments¹²⁻¹⁴ lycopene and β -carotene in intact plant samples (Figs. 1a and b). Resonance enhancement is obtained in the Raman spectra of pigments excited at wavelengths in the region of electronic absorption. The effect is manifest in a conspicuous gain in the scattering efficiency from some of the vibrational modes of the pigment. The scattered light becomes sufficiently intense to overcome the increased absorption losses of both the exciting and the scattered radiation. In this study we have demonstrated this effect in heterogeneous (tissue) samples, in which the pigment is just one constituent.

Spectra observed at different excitation frequencies have confirmed the sideband character of the emission lines, so that perhaps the name rigorous resonance Raman effect (RRE)¹¹ more adequately describes our findings¹⁵. Considerations of whether RRE at peak absorption differs from resonance fluorescence are sidestepped¹⁶⁻¹⁸.

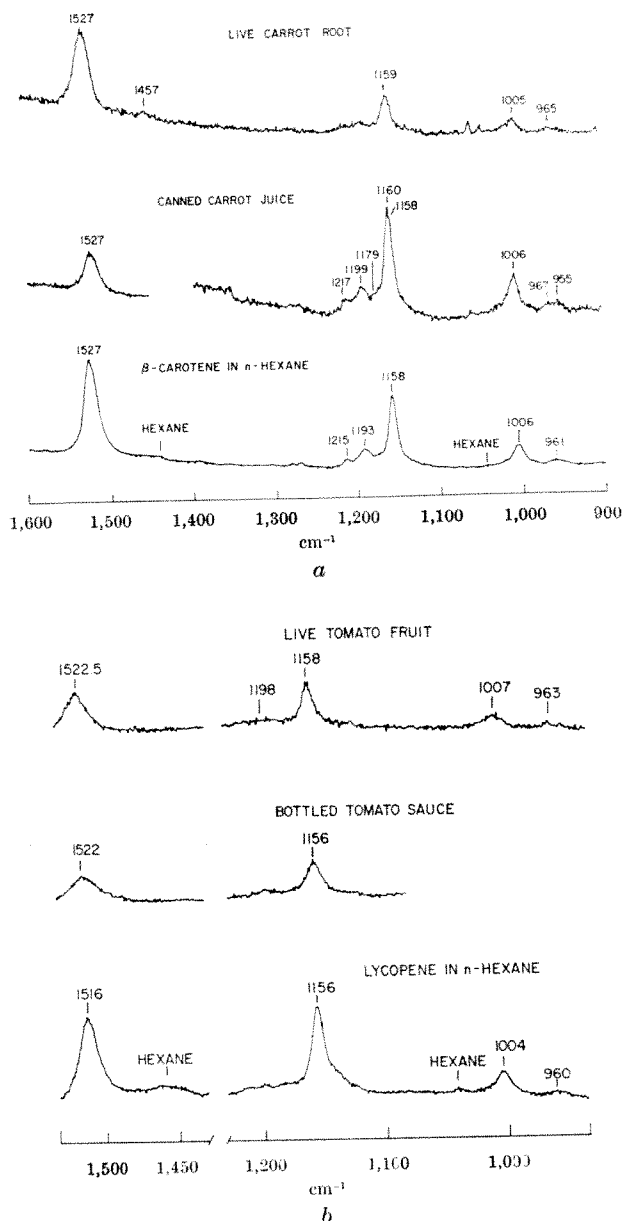


Fig. 1. Vibrational fundamentals in the resonance-enhanced Raman spectra of: (a) β -carotene in live carrot root, in canned carrot juice and the *n*-hexane solution of the pure all-*trans* pigment (Sigma) (excited at 488 nm); (b) lycopene in live tomato fruit, in bottled tomato sauce (the 1527 line of the canned carrot juice spectrum was taken with the gain reduced by a factor of 2) and solutions of lycopene extracted from bottled tomato sauce¹⁹ in an *n*-hexane solution (excited at 5,145 nm). Excitation by argon-ion laser, incident power ~ 100 mW. Grazing angle reflexion geometry: the electric vectors of incident and of analysed scattered light are both perpendicular to the scattering plane. Spectrometer: 75 cm Czerny-Turner double monochromator, digital photon counting detection, integration time 1 s, scanning speed 20 $\text{cm}^{-1}/\text{min}$.

The remarkably intense RR spectra reported for conjugated polyenes in solution¹⁹⁻²² and our study of Raman excitation profiles in carotenoid solutions¹⁵ suggested that RR scatter from pigment molecules in biological samples would be observed without interference from background scattering. Maximum enhancement is obtained in carotenoids, the first absorption band of which is centred within the spectral range of the argon laser emissions. Before the work on live tissues was commenced, light at any one of the frequencies available from the argon-ion laser was scattered from solutions of the pigments in *n*-hexane. By using the Raman lines of hexane as internal standards of intensity, the excitation profiles of the pigments in solution were determined, yielding the wavelengths for optimal excitation¹⁵.

The laser beam impinged at a grazing angle on the surface of the sample facing the collector lens. This applied to solid and fluid samples alike²³. The scattered light was resolved in a double monochromator, then detected in a photomultiplier and processed in digital photon counting circuitry. We have examined the samples listed in Fig. 1. The surprisingly intense RR spectra from the tissue samples give information on the pigment molecules and on their environment. The information content of RR spectra is different and probably greater than that of absorption or luminescence spectra because different selection rules are obeyed. The RR-active chromophore can therefore be added to the list of biophysical reporter groups²⁴⁻²⁶.

We summarize here some of the results and conclusions from the work on the live samples (excitation profiles and spectra in solutions are reported elsewhere)¹⁶. First, RR spectra of carotenoid pigments can be readily obtained by scattering laser radiation of appropriate wavelength from live tissue samples. Second, the Raman lines observed are listed below (the $\nu_1, \nu_2 \dots$ notation for fundamentals was adopted from Shorygin *et al.*²⁰⁻²² and extended beyond ν_3). The frequencies corresponding to vibrational modes are given in wavenumbers. Unbracketed frequencies refer to solutions in *n*-hexane; bracketed frequencies refer to tissue samples. For lycopene: $\nu_2 = 1516$ (1522.5); $\nu_1 = 1156$ (1158), ν_1 satellites = 1210, 1200, 1185 (1198); $\nu_4 = 1004$ (1007); $\nu_5 = 960$ (963); $\sim 2\nu_2 = 3030$ (3045); $\sim (\nu_1 + \nu_2) = 2667$ (2672); $\sim 2\nu_1 = 2300$. For β -carotene: $\nu_2 = 1527$ (1527); $\nu_1 = 1158$ (1158), ν_1 satellites = 1215, 1193, 1176 (1217, 1194, 1176); $\nu_4 = 1006$ (1006); $\nu_5 = 961$ (965); $\sim 2\nu_2 = 3048$ (3046); $\sim (\nu_1 + \nu_2) = 2679$ (2680); $\sim 2\nu_1 = 2313$ (2310); $\sim (\nu_1 + \nu_4) = 2163$.

Third, the spectra are rich in harmonic and combination bands, only some of which are reported here. The most intense ones involve the frequency ν_1 . Fourth, significant vibrational frequency shifts have been observed between the hexane solutions and the live tissue and canned samples. We suggest that these shifts are the result of solvent effects and believe that, by studying model compounds, inferences could be drawn on the local environment of the pigment in the live tissue. Fifth, the line widths and shapes for the live samples differ from those for the canned preparations. The fundamental ν_2 is especially sensitive. Sixth, the O-H stretching vibration in water is observable in the live and the canned samples as a broad shoulder centred at $\sim 3,600 \text{ cm}^{-1}$.

The concentration of pigments in these samples is very high, but RR spectroscopy in solution is known to be sensitive to submicromolar concentrations of pigment and the same probably applies to biological samples. Unfortunately, quantitative determinations are extremely difficult in the absence of internal reference lines.

Laser irradiation of pigments in the absorption region cannot be termed non-destructive, and obviously the chemical and isomeric purity of the carotenoids is somewhat impaired. In practice we have observed no deterioration in the Raman and the absorption spectra of the solutions in *n*-hexane after 1 h of laser irradiation. Moist plant tissue like the inner sections of the tomato fruit or fresh carrot endured the radiation better than a half-dry carrot or autumn leaf.

We thank Mr J. L. Parsons and Dr T. Cole of this laboratory and Professor S. Yatsiv, Professor M. Calvin, Dr M. P. Klein, Mr R. A. Bogomolni, Professor Y. R. Shen and Professor B. Rosenberg for their advice.

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Algae thrive under Pure CO₂

ATTEMPTS to grow photosynthetic organisms (O₂ producers) in CO₂ have usually used CO₂ as part of a mixture with other gases. Greater concentrations of CO₂ are toxic to plants and decrease their photosynthetic levels through narcotic poisoning or acidification of the cell fluids¹. We do not know any report of plant growth and physiological activities in anaerobic conditions with pure CO₂. The relation of photosynthesis to environmental conditions such as a pure atmosphere of CO₂ and light intensity is of practical interest in, for example, space biology.

We have carried out physiological experiments with the unicellular hot spring acidophilic alga *Cyanidium caldarium* cultured in pure CO₂. The alga was grown in 500 ml. culture flasks with built-in gassing tubes. A double strength of the mineral medium described by Allen², adjusted to pH 2-3 with sulphuric acid, was used in all experiments. The growth flasks were continually agitated (Burrell, wrist-action shaker) inside a hot water bath covered with a thin layer of mineral oil. The temperature was adjusted to 45° C, which is within the optimal range found both in nature³ and in the laboratory⁴. Continuous illumination was provided by eight General Electric cool white fluorescent tubes supplying an intensity of about 450 foot candles. The CO₂ (commercially available by Liquid Carbonic Corp.) was bubbled at a flow rate of about 200 ml./min and was humidified by passage through water. For each CO₂ flask an air control culture was grown (twice filtered laboratory pipe source); otherwise both were treated similarly. Growth value was determined by measuring the packed cell volume in graduated Hopkins centrifuge tubes.

Photosynthetic determinations were made with a 3-5 ml. oxygen electrode chamber which is described by Shugarman and Appleman⁵. The cells used for photosynthetic studies were harvested about 1 h before the measurements were made. Algae cells were flushed for about 5 min with N₂ to remove all dissolved O₂ in the suspension and then incubated at 28°-29° C and illuminated at 4,500 foot candles.

Illustrations for growth and photosynthetic rates of *Cyanidium* grown under CO₂ or air are shown in Fig. 1.

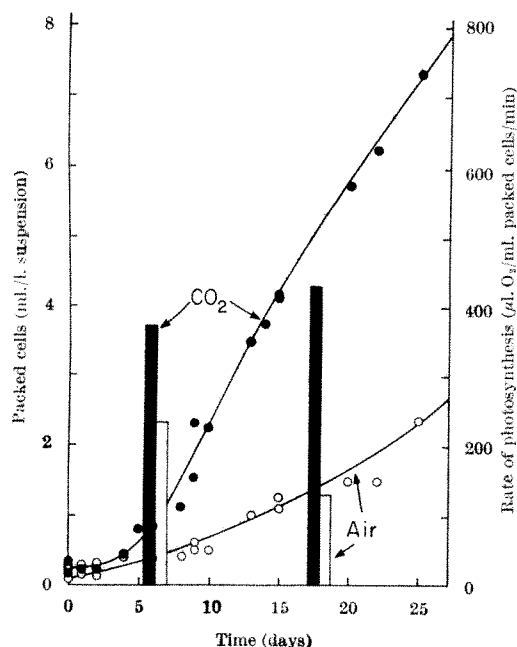


Fig. 1. Growth of *Cyanidium caldarium* (circles) and the photosynthetic rates (histograms) as a function of CO₂ (solid figures) or air (open figures) supply to the cells.

Both the volume of the packed cells and the O₂ production are significantly higher in the CO₂ treated cultures. The growth curves have a low increase of cells during the first few days, then the CO₂ culture shows a marked increase in the growth slope while the air control grows more slowly but with a steady increase. The adaptation period for this alga under pure CO₂ may decrease the growth rates during the first days. The total O₂ production measured after 18 days of CO₂ treatment is more than that of the 6 day culture. Control cells grown under air have more O₂ per packed cells when the culture is younger (6 days) and less in the older culture (18 days).

The evidence for accelerated growth and photosynthesis under pure CO₂ is noteworthy. The optimal growth for *Cyanidium* occurs in relatively low light intensities (350 to 600 foot candles), as reported elsewhere^{6,7}. Allen² stated that when *Cyanidium* was grown aerobically at high light intensities, it lost some pigments and the culture turned yellowish-green. We observed that the photosynthetic rate rises progressively with the increase of light intensity in treatments under CO₂ or air. *Cyanidium* grown on CO₂ or air is still active in O₂ production at great light intensity (more than 7,000 foot candles) and these photosynthetic rates are more elevated than at a smaller intensity. Similar increasing rates of photosynthesis with light intensity in *Cyanidium* were reported by Fukuda⁸, who found the saturation CO₂ concentration (3 per cent) at about 10⁵ lux (about 9,300 foot candles). The *Cyanidium* cells were obtained from Yellowstone hot springs where they grow at a maximum temperature³ of 56° C. Observations by Copeland⁹ suggested that *Cyanidium caldarium* grows at temperatures of 80° C or above, which contradicts another report³ and also our investigations (unpublished data). A gas analysis of the spring volcanic steam showed that, except for water, CO₂ is the main constituent and is more abundant than all the other gases combined¹⁰. We did not add any sugar or other organic compounds to mineral media, and the pure CO₂ cells were grown in a solution saturated with CO₂, while the aerated culture (without supplemental CO₂) could possibly be affected because of a CO₂ deficiency. It was established that this eukaryotic cell (*Cyanidium*) can be adapted to grow in pure CO₂ while the prokaryotes tested (thermophilic blue-green algae) are unable to sur-

vive such treatment. This indicates that the toxicity of CO₂ is a special problem in virtually every case.

We thank all who showed an active interest in the project, especially Dr W. F. Libby; and W. N. Doemel and Dr T. D. Brock for the *Cyanidium* cultures, Dr R. W. Castenholz for the thermophilic blue-green algae and Dr C. A. Schroeder for his equipment. This research was supported by NASA and the US Air Force Office of Scientific Research.

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Mutants in Phototaxis of *Dictyostelium discoideum*

ONE of the more interesting behavioural characteristics of the cellular slime mould, *Dictyostelium discoideum*, involves the concerted migration of thousands of amoebae in a pseudoplasmodium or slug towards light. There have been several studies of the phenomenon, and the action spectrum for phototaxis has been shown¹⁻⁴ to have peaks at 425 and 550 nm. The biochemical process by which light energy controls the direction of movement, however, remains unknown. Moreover, the mechanism of locomotion which can carry a 1 mm long slug for several centimetres in a period of 2-3 days is unclear, but seems to involve more than directed amoeboid motion of the individual cells. Because the amoebae in a slug move through a casing of slime secreted as a viscous fluid by the tip cells⁵, it seems probable that both the extent and the direction of migration would be dependent on the rate of formation of slime casing. Because further biochemical analysis might benefit from the availability of mutants which lack the phototactic response, conditions which would make it possible to select such mutant strains were developed.

Single drops of an overnight SM broth culture of *Aerobacter aerogenes*⁶ on 2 per cent washed agar were found to allow inocula of a few *D. discoideum* cells to grow to populations of 5 × 10⁵ to 2 × 10⁶ amoebae. When the amoebae depleted the bacterial food supply, they aggregated and formed up to twenty slugs. If the amoebae were incubated in a light-tight container (a baking pan with aluminium foil was found convenient), the slugs migrated randomly, leaving the depleted drop of bacteria at all points over the 360° of the circle. If, however, visible light was allowed to enter the container through a small hole, the slugs oriented themselves and migrated within an arc of about 30° directly towards the light.

To enrich the population in mutant strains, wild-type cells were treated with the mutagen N'-methyl-N'-nitro-



Fig. 1. Phototactic response of *D. discoideum* strains. Droplets containing 10^7 washed cells of strain NC-4 wild-type (left drop), strain L-20 (centre drop), strain L-25 (right drop) were placed on an agar base in a 9 cm Petri dish and exposed to a point source of light (bottom centre of photograph). After 48 h incubation at 22° C the cells had aggregated to form slugs which migrated out of the drops. The path taken by each slug is clearly marked by slime excreted along the way. The slugs, which are about 1 mm long, can be seen at the ends of the slime trails. Photographed by transmitted light.

N-nitrosoguanidine⁷ and then spread on normal growth plates with bacteria. After incubation for 4 days at 22° C, individual clones of amoebae were picked and inoculated into bacterial drops on a 2 per cent agar base in a light-tight container with three small holes at one end. After incubation in a normally illuminated room at 22° C for 4 days, the isolates were inspected for phototaxis. In all, 614 such isolates were screened. Most of the isolates formed slugs which migrated directly towards the light. Several failed to form slugs while others formed slugs which failed to migrate at all. Two isolates formed normal slugs which then left the bacterial drop over a large sector and migrated in an essentially random manner similar to that of wild-type slugs migrating in the dark. These strains then constructed fruiting bodies which accumulated yellow spore pigment normally. The strains L-20 and L-25 were subcultured and studied further.

Although phototaxis in strain L-20 was clearly abnormal in the above conditions, many slugs of this strain ultimately oriented towards the light source. To determine whether more intense light could increase the orientation, 10^7 washed cells of strains NC-4 (wild-type), L-20 and L-25 were deposited in small drops on an agar surface in a Petri dish painted black except for a small hole. A strong beam of light from a microscope illuminator was directed at the hole about 5 cm away. The wild-type cells formed slugs which migrated directly towards the light while slugs derived from strain L-20 again oriented poorly towards the light (Fig. 1). Slugs derived from strain L-25 migrated in total disregard to the direction of the intense light source.

The pattern of migration seen in Fig. 1 gives the impression that the extent of migration is much smaller in slugs of the mutant strains than in those of the wild-type strain and suggests that the mechanism of locomotion might be impaired in the mutants. It must, however, be remembered that slugs of strain L-25 proceeded on a random walk while those of wild-type NC-4 moved straight towards the light. Slugs of strain L-20 moved on an intermediate path. When the lengths of the slime trails of the mutant strains were followed over their crooked courses, it was found that the slugs had migrated at least half as far as those of the wild-type. It was concluded that the slugs of the mutant strains not only have apparently normal morphology but are also able to migrate in

an essentially normal fashion except for an impairment in phototaxis.

It has been known for several years that small numbers of wild-type cells can interact synergistically with morphological mutant cells of *D. discoideum* to form normal fruiting bodies^{7,8}, and it therefore seemed possible that wild-type cells might lead cells of the blind mutants towards the light. When cells of strain NC-4 were mixed in equal portions with cells of strains L-20 and L-25, however, the phototactic response was only slightly improved. The response seems to be essentially restricted to the individual cells and not transmitted throughout the organism.

Phototaxis in *D. discoideum* has been shown to be effected by the "lens effect" of the translucent cells³. It seems that light is focused on the side of the slug away from the light and preferentially stimulates these cells. It is thus quite possible that these cells are stimulated to produce more of the slime casing and thus turn the slug towards the light.

The isolation of photo-insensitive mutant strains of *D. discoideum* proves that at least certain behavioural attributes of this organism are under direct genetic control. Further studies involving these mutant strains may elucidate the chemical basis of phototaxis.

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Lower Cretaceous Fleas

Two fleas have been collected in association with abundant remains of fish, plants, phyllopod Crustacea (Conchostraca, Anostraca and Cladocera) and other insects in a Lower Cretaceous siltstone at Koonwarra, southern Gippsland, Australia. The insect fauna consists mainly of the aquatic immature stages of Ephemeroptera (mayflies), Diptera (midges), Coleoptera (beetles), Plecoptera (stoneflies) and Odonata (dragonflies and damselflies). The aquatic larvae of the scorpionfly family Nannochoristidae (Mecoptera) are also preserved. There is also a small adventitious fauna of adult insects, including the fleas, the immature stages of which are not aquatic. The only known remains of warm-blooded vertebrates are feathers¹.

One flea is of normal pulicid form and size. In many respects it is ancestral to the modern stickfast fleas (*Echidnophaga*), some species of which are endemic on Australian marsupials. This flea has very elongated mouthparts similar to those of the stick-fast fleas.

The second flea (Fig. 1) is most interesting, for it combines a typical flea abdomen and male genitalia with a very primitive head structure. The head is less compressed and the antennae are not markedly shortened and recessed in grooves along the side of the head as in modern fleas; they are prominent although the segments are short and of cone-in-cone form. The legs are unusually long and the tibiae bear pseudocombs of short stout

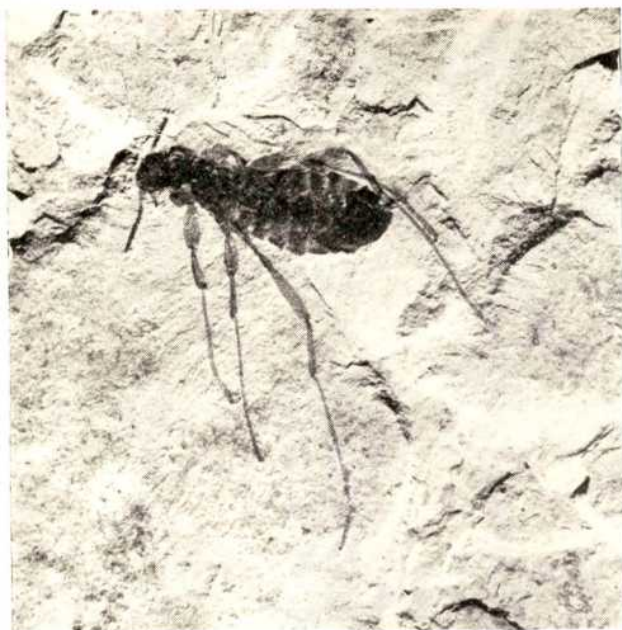


Fig. 1. One of the Koonwarra fleas.

bristles: the hind femur is not enlarged for jumping. The structure of the legs suggests that this insect lived on a sparsely haired (furred) animal and that it clung to the outer portions of the hairs rather than burrowed between the hairs. The specimen, with a body length of 7 mm, is large as compared with most modern fleas, especially as it is a male: male fleas are distinctly smaller than females. Females of some modern fleas, however, are of this order of size. The nematocerous-type antennae tend to support the more usually accepted conclusion that the fleas evolved from a nematocerous-type ancestor.

The presence of two very different types of fleas or flea-like insects in the Lower Cretaceous, one of which is similar to modern fleas, indicates that the Siphonaptera must have had a long history before the Lower Cretaceous. The more primitive of the two species shows that loss of wings and development of a specialized copulatory mechanism occurred before reduction in nematocerous-like antennae resulted in their being recessed in grooves on the head, and before the development of combs on the head and thorax. The Siphonaptera must have arisen from a primitive nematocerous-like ancestor before specialization of the male terminalia resulted in structures similar to those of modern Nematocera. Undoubted Diptera are recorded from the Triassic. It seems probable that the Siphonaptera evolved from nematocerous Diptera in the Late Triassic or early Jurassic, at about the same time as the first warm blooded vertebrates.

Deductions concerning the ecological association of at least the more primitive of the two species with a furred animal, and not a bird, indicate that marsupials must have been present in Australia at a very much earlier period than has hitherto been conceded, and this species thus sheds new light on the probable centre of origin, and on the early dispersal of the marsupials as it affects the zoogeography of the southern continents.

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Taxonomy and Cranial Capacity of Olduvai Hominid 7 (continued)

PILBEAM rejects¹ my recent estimate of cranial capacity for *Homo africanus*². As I understand his comment, he disputes treatment of Olduvai hominid 7 with the South African gracile australopithecines as one statistical population because they may belong to separate subspecies.

The question of how many subspecies may exist within *Homo africanus* was never raised because it is not relevant. Given the possible time span of this taxon, and the nature of many hominid-bearing deposits, the only safe assumption would be that each specimen represents a distinct biological population³. It is for precisely this reason that species are generally the smallest meaningful taxonomic unit for fossil material⁴. When cranial capacity is considered as a species parameter, the specimens representing the species make up the statistical universe. It seems to me that estimations of species parameters are best made if all of the specimens referred to the species are considered. Indeed, Pilbeam seems to contradict himself, supporting the inclusion of Olduvai hominid 7 in "*africanus*" on the basis of cranial capacity, in a paper⁵ published the same month as the comment considered here¹ (October 1969).

It may be added that the use of a *t* test for small sample sizes is misleading. It is not unusual to find relatively high *t* values for the large or small ends of a small sample. For instance, using the published data for *Homo erectus*⁶, the *t* value calculated for the largest *Homo erectus* cranium (skull 10 from Choukoutien) is 2.93 (10 degrees of freedom). This indicates a probability of 0.98 that the largest *Homo erectus* cranium does not differ from the eleven other crania by chance alone. It does not place skull 10 in another species, let alone another genus.

In fact, this result does not even mean that skull 10 is necessarily representative of a separate biological population. The parameters which characterize taxa, whether on subspecies, species or supraspecies levels, can only be derived from the distribution of the actual specimens⁶.

In sum, Pilbeam's criteria for drawing firm taxonomic conclusions have been met in the case of Olduvai hominid 7. There is no morphological evidence in either the dentition or the cranial capacity which justifies, or even suggests, the separation of this specimen from *Homo africanus*.

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Early Hominids and Cranial Capacities (continued)

I SHOULD like to make a few points arising from the previous letter¹. Much of the debate centres around the problem of species in palaeontology; how to define these, and how to assign new specimens to their appropriate taxon². It is obvious that "the specimens representing the species make up the statistical universe". The point which Wolpoff ignores is the manner in which individual specimens are assigned to "species". In classifying fossils, due attention must be paid to variation within and between infraspecific populations, as well as to possible variation due to time³. The most important issue to decide in the

evaluation of a new find is the species lineage (that is, a sequence of ancestral/descendant species populations) to which it belongs, and this requires some consideration of position with respect to infraspecific lineages (infraspecific lineages being sequences of ancestral/descendant infraspecific populations which are not isolated from others but which do have some time duration). Only when this decision has been made ought attention to be turned to classification at the species level, that is, to dividing up the evolving continuum and giving the arbitrary divisions binomina.

Although the dating of the South African *Australopithecus africanus* sites (Taung, Sterkfontein and Makapans) is far from accurate, there is some agreement that they are sampling a time period in excess of 2 million years³. Although it is obvious that each specimen represents a population, this is not the point I was trying to make. The specimens from Sterkfontein, for example, probably cover a relatively short period of time, and it is very likely that they are sampled from one infraspecific lineage of hominids living in that area. In the parameter under consideration, cranial capacity, they show only a small range of variation, four specimens⁴ varying between 428 cm³ and 485 cm³. It is generally agreed that Makapans is a little younger than Sterkfontein. One estimate of cranial capacity is available from Makapans, 435 cm³. Morphologically, the specimen is very similar to those from Sterkfontein⁵. The status of the Taung infant, of uncertain geological age, is more equivocal, but at present it can be assumed that it is sampled from the same species lineage, and very probably the same infraspecific lineage; its inferred adult cranial capacity⁴ is 440 cm³. The range for the six values for *A. africanus* is 57 cm³ (428–485), and the coefficient of variation (corrected for sample size) is only 5.2.

The same taxon, *A. africanus*, is apparently represented in deposits in the Omo region of Ethiopia which range in age from a little under 2 million to a little under 4 million years⁶. At present, reported material consists only of teeth and jaws. There are only minor differences between the Omo and South African samples (particularly Sterkfontein) and it seems reasonable to assume that the samples represent geographical variants of the same species lineage drawn from approximately the same period of late Cenozoic time. It is also reasonable to include all specimens within the same arbitrary segment (palaeospecies) of this lineage, and describe them all as *A. africanus*⁶. (I prefer this generic designation to *Homo africanus* for reasons outlined elsewhere⁷.)

In considering the position of Olduvai hominid 7, the first question to ask is "To which lineage does the specimen belong?". The age of this specimen is known (approximately 1.75 million years), and it is therefore younger than both Omo and, if the assumptions are correct, the three South African sites. Dental, gnathic and postcranial evidence suggests that hominid 7, together with other specimens from Olduvai Bed 1 which apparently sample an East African infraspecific lineage younger than 2 million years old, are drawn from a later part of the same species lineage as is represented at Omo, Sterkfontein,

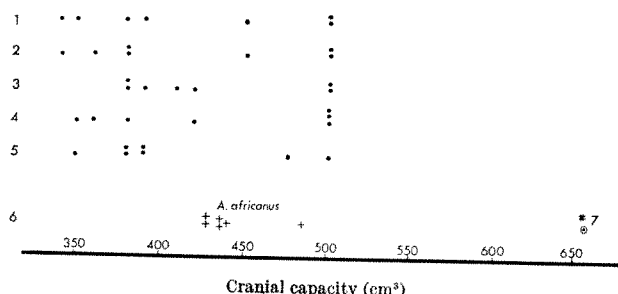


Fig. 1. Samples 1 to 5 are drawn at random from a group of twenty chimpanzees. Sample 6 shows the distribution of *A. africanus* and hominid 7 from Olduvai Bed 1.

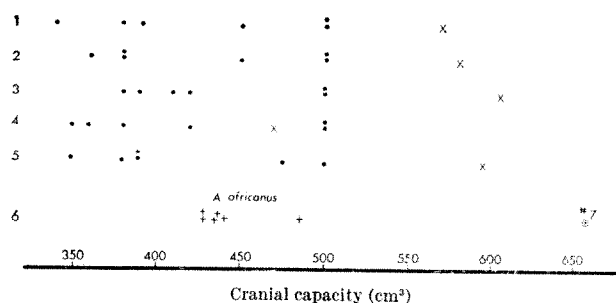


Fig. 2. Samples 1 to 5 are taken from a group of twenty chimpanzees (dots) and twenty gorillas (crosses). Sample 6 shows the distribution of *A. africanus* and hominid 7 from Olduvai Bed 1.

Makapans and Taung. There is a distinct possibility though that there has been evolutionary change in this lineage in at least one parameter, cranial capacity, as can be seen by comparing the value for hominid 7 (657 cm³) with those from South Africa (\bar{x} = 442 cm³, s = 22 cm³, o.r. 428–485 cm³, V_{cor} = 5.2, for n = 6)⁴. Using the t test, the only appropriate statistical test available, hominid 7 differs very significantly from the South African sample⁴ (contra statements in ref. 7, which were based on earlier and less accurate volume estimates^{8,9}). Other cranial capacity estimates are available for Olduvai hominids younger than 2 million years, and give values of at least 600 cm³. It would seem, on admittedly sparse evidence, that the differences are unlikely to result from sampling error, but rather reflect evolutionary change in this parameter.

There is another, more empirical approach which can be taken. The differences between hominid 7 and the six *A. africanus* estimates might arise from sampling error. Accordingly, I have taken a series of twenty male and female chimpanzees of known cranial capacity and, using random numbers, extracted samples of seven individuals and examined the pattern of distribution of these. Twenty such samples were taken, and a random selection of five of these distributions is shown in Fig. 1. None of them resembles the pattern exhibited by Olduvai hominid 7 and the six *A. africanus* specimens. A similar procedure was adopted in which six individuals were selected at random from the chimpanzee group together with one individual from a group of twenty male and female gorillas. Twenty such samples were taken, and five of them are shown in Fig. 2. These distributions resemble that of the fossil hominids rather more than do the samples of chimpanzees only. Hominid 7 still appears, however, to differ more from the six known *A. africanus* than does any one gorilla drawn at random from any random sample of six chimpanzees. Once again the evidence would indicate that, on the basis of cranial capacity, hominid 7 differs as much from earlier hominids as does the gorilla from the chimpanzee. It would seem most reasonable, therefore, not to include hominid 7 in *A. africanus*, but to refer it to another species of the genus *Australopithecus*⁷.

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¹ Wolpoff, M. H., *Nature*, **227**, 747 (1970).

² Simpson, G. G., *Principles of Animal Taxonomy* (Columbia Univ. Press, 1961).

³ Howell, F. C., *Amer. J. Phys. Anthropol.*, **27**, 95 (1967).

⁴ Holloway, R. L., *Science*, **168**, 966 (1970).

⁵ Tobias, P. V., *Olduvai Gorge*, **2** (Cambridge Univ. Press, 1967).

⁶ Howell, F. C., *Nature*, **223**, 1234 (1969).

⁷ Simons, Elwyn L., Pilbeam, David, and Ettel, P. C., *Science*, **166**, 258 (1969).

⁸ Tobias, P. V., *S. Afr. J. Sci.*, **64**, 81 (1968).

⁹ Robinson, J. T., in *Evolutionary Biology*, **1** (edit. by Dobzhansky, T., Hecht, M. K., and Steere, W. C.), 69 (Appleton Century Crofts, New York, 1967).

Book Reviews

DIET IN VICTORIAN TIMES

The Dietary Surveys of Dr Edward Smith 1862-3

A New Assessment. By T. C. Barker, D. J. Oddy and John Rudkin. (Occasional Paper No. 1.) Pp. 62+3 plates. (Staples, for the Department of Nutrition, Queen Elizabeth College, London: London, March 1970.) 12s 6d.

THIS is the first of a series of papers from an interdisciplinary group which meets three or four times a year at Queen Elizabeth College, London, to discuss the development of diet and food habits in this country. This collaboration between economic historians, social scientists and nutritionists will, it is hoped, throw more light on why people eat what they do, and the effect on their health. An assessment is made of the nutritional value of the diets Dr Smith collected in the early 1860s, comparing them with Boyd Orr's findings in the 1930s and with the position for lower income groups in 1965. Some conclusions are drawn on the relationship of particular dietary features, such as the intake of sugar, fat and calcium, with certain diseases. The study begins and ends with comments on the life and career of Dr Edward Smith himself, and a bibliography of his publications.

Listed in this way this work sounds impressive. Yet it leaves the reader dissatisfied and with an unfortunate impression of being too slight. Perhaps this is because the most interesting questions cannot be followed up in this publication. The authors' purpose will be served, however, if their work creates further interest in an analysis of historical data using modern techniques to assess the quality of the diet consumed.

Dr Smith seems an interesting man, another of those public spirited Victorians who found his environment wanting, especially for others less fortunate than himself. He was an uncomfortable ally and it would be interesting to know more of the nature of the quarrel he had with Sir John Simon, whose department under the Local Government Board created in 1871 absorbed Smith's work as Medical Officer to the Poor Law Board.

There are some aspects of the comparative sections of this discussion on Smith's survey which appear hasty and incomplete—the comparison, for example, between the wage material collected by James Caird in 1850-51 and Smith's data on the rural labourer. The limitations of Smith's own work are, however, more carefully explained, especially its coverage of certain types of indoor workers—silk-weavers, needlewomen, glovers and shoemakers, as well as some rural labourers. He also interviewed cotton workers during the depression of the early 1860s, and compared their diets with those they had enjoyed before the American Civil War. The nutritional analysis is done with the same care; the calories and nutrients have been calculated by computer from tables of food composition, making allowances for differences between nineteenth and twentieth century foods, such as bread. The vitamin content has not been measured because of the hazards of various processes of preservation and cooking. On the other hand, some comment on the possible deficiencies in vitamin intake would have been helpful in an assessment of diets containing so few vegetables other than potatoes and little fruit.

The indoor workers in Smith's sample, excluding the

cotton workers, seem to have enjoyed a poorer diet than rural labourers, especially in calories, protein, iron and calcium. In the past hundred years there has been a large fall in bread consumption resulting in a reduction in calories, but compensated for by a rise in consumption of milk, meat, fat and potatoes. There are difficulties in assessing the way in which foods were distributed between members of the family, but the authors conclude that the quality of diet in the 1860s must have affected work efficiency adversely.

Some interesting observations are made on the effect of a reduction in family income on diet. Cuts in expenditure during the cotton famine were made on all foods, and not just on the quality items, largely to maintain palatability, it is suggested. Yet the reductions in meat and fats were particularly severe, and bread was rendered palatable by spreading more treacle on it. The retention of a high level of sweet substances particularly interests the authors, as does the very large increase in sugar consumption of the past hundred years and the possible connexion between this and coronary thrombosis. Their other comments on the relationship between diet and disease concern the calcium intake; yet it seems rather a lavish claim from the evidence given in this publication to suggest that the low intake of calcium in the 1860s among the cotton workers was not matched by any clinical evidence of calcium-deficiency diseases. It seems that the general effect of the impoverished diet of the mid-nineteenth century, made worse by the cotton famine, was an impairment of resistance to infectious diseases such as typhus, whooping cough, chicken pox, scarlet fever, and possibly to diseases such as tuberculosis.

JANET BLACKMAN

SEEKERS OF NATURE

The Eternal Quest

The Story of the Great Naturalists. By Alexander B. Adams. Pp. 509+12 plates. (Constable: London, June 1970.) 70s.

ANY attempt to survey the progress of knowledge in the natural sciences is a formidable undertaking. Within the confines of a single volume it represents an exercise in selection and compression, which inevitably invites criticism for omissions or emphasis. Mr Alexander Adams succeeds in his declared objective of writing the story of man's discovery of his place in the natural world and to show the qualities and characters of some of the men who made these discoveries.

The Eternal Quest opens with Aristotle, his teachings, especially his observational approach, and critical appraisal of all scientific concepts, and strangely Pliny the Elder, arch-compiler and encyclopaedist of the first century AD. Succeeding essays tell in narrative of the contributions of Copernicus, Vesalius, Bruno, Kepler, Newton and others to the progress of scientific thought. With a more detailed review of the work of Linnaeus the emphasis turns to the naturalists, in the modern meaning of the word. Here the acclaim given to Linnaeus's system of classification is probably overdone. Great as Linnaeus's contribution to natural history was, his classification and the concept of binominal nomenclature owed much (perhaps more than he acknowledged) to John Ray and Peter Artedi. Both are mentioned in this book, but their contribution to the development of Linnaeus's method is surprisingly not.

From Linnaeus the book turns to the work of Buffon, Lamarck, Cuvier, and the latter's pupil Louis Agassiz, whose early work on glaciers and fossil fishes led him to a position of great influence especially in the United States. The life and work of Alexander Wilson, the "father of American Ornithology", son of a Scottish smuggler, and himself a forced emigrant from Scotland who, although

desperately poor all his life, devoted his slender resources, great energy and genius to the production of his *American Ornithology*. Audubon, his successor, is naturally included (for Adams is the author of a definitive study, *John James Audubon* (1967)). Humboldt, Lyell, Darwin, Wallace, Huxley and Mendel bring the history up to the beginning of the present century.

The personalities of these naturalists were so different that this study makes a fascinating human document. Some were modest, some arrogant, but all were driven by an invisible demon of discontent with accepted ideas, and were therefore continually searching for a tenable position. Whatever one's personal quibbles over the omission of some favourite naturalist, Adams's *Eternal Quest* is a "wide screen" treatment of the development of natural history, giving a balanced and broad picture of absorbing interest to the general reader and an introduction to the subject.

ALWYNE WHEELER

SCIENCE FOR SCHOOLS

Nuffield Combined Science Pack 1

Activities—Book 1: Pp. 72; Book 2: Pp. 66; Book 3: Pp. 40; Book 4: Pp. 47; Book 5: Pp. 36. Reference Sheets: Pp. 27. Teachers' Guide 1 (Sections 1–5): Pp. xxiii + 411. Teachers' Guide 3: Pp. vii + 266. (Longman: London; Penguin: Harmondsworth, Middlesex, 1970.) n.p.

THIS is the first batch of materials for use with the new *Nuffield Combined Science* course for 11 to 13-year-olds. *Teachers' Guide 2* and five more *Activities* booklets will complete the set.

Any attempt at really integrating the various branches of scientific knowledge so that the product is satisfying for both teacher and taught faces major problems. These problems are concentrated in the selection of material and its presentation in such a way that the users do not get the feeling of hopping haphazardly from one topic to another. In general, the four authors of this course can feel reasonably satisfied with their efforts at coherence and clear presentation. It is instructive to compare the Nuffield course with two other attempts at integration: the *Science Worksheets* prepared by the Scottish Secondary Science Working Party (published by Heinemann Educational Books), and Professor Harry Messel's *Science for High School Students* (University of Sydney). There are many similarities in the outlook and approach of the three groups and also in their choice of experimental and illustrative material.

The important thing about any new course is how the consumers react to it. The trials for this one involved thirty-five schools, eighty teachers, and three thousand children; and, making an allowance for the inevitable Hawthorne effect, there is good evidence that the materials were well received. *Teachers' Guide 1* quotes many comments (not all of them complimentary) from the teachers involved. The authors claim that the course can be adapted for use with the whole range of ability, and there is a useful appendix consisting of comments from teachers who used it with slow learners.

The authors of the *Teachers' Guides* successfully walk the tightrope between appearing to be dogmatic and definitive (so cramping the originality of the teacher), and failing to give adequate guidance to those many teachers who will have to deal with topics outside the range of their personal knowledge. *Teachers' Guide 3* is a reference book: full of useful hints, details of apparatus, materials, and teaching aids, with a mathematics appendix. *Teachers' Guide 1* ties in with the *Activities* booklets for the pupils. Both *Guide* and booklets are well illustrated, and the instructions are detailed without being fussy. The booklets are clear and readable, but will probably have to be treated as expendable. The *Guide* contains

some excellent educational philosophy; the introduction to the section on "Looking for Patterns" is particularly impressive. There is a welcome emphasis on doing simple experiments at home; and in places one can detect the influence of that seminal classic, UNESCO's *Source Book for Science Teaching*.

There are three major limitations to curriculum innovation in science: the capacity of the teachers, the time allocated within the school curriculum, and money. This particular course is intended to be taught by one teacher throughout. I, for one, could not do it without an extensive period of in-service training; for example, how many physics and chemistry teachers would back themselves to dissect a rat properly, even with full printed and photographic instructions? Also, there is currently an absolute shortage of science teachers, particularly for the physical sciences (*vide* the Royal Society's recent report). The Nuffield group suggests that this course should occupy five periods per week. I would regard this as an absolute minimum; but many headmasters and more particularly headmistresses would think it too much, especially for the first year. The authors also warn that the costs of the *Nuffield Combined Science* are likely to be higher than for some other schemes.

All those concerned with the teaching of science in the early secondary years should give serious considerations to these books.

MARTYN BERRY

ISLAND RIDDLES

Serendipity in St Helena

A Genetical and Medical Study of an Isolated Community. By Ian Shine, with the assistance of Reynold Gold. Pp. xv + 187. (Pergamon: Oxford and New York, 1970.) n.p.

EPIDEMIOLOGISTS are said to plan their studies by first defining their population, and there are few studies whose success has turned upon the chance discovery of a population. Such, however, was the good fortune of the author of this monograph who found himself, by chance, medical officer to a remarkable island population. The ancestors of St Helena's population today were Europeans, Indians, Africans and Chinese who arrived voluntarily or by force and who have interbred over the past three hundred years to a remarkable extent. The 4,642 men, women and children who live there today consequently form a uniquely interbred and even inbred mixture of races in whom the pattern of disease might be expected to be unusual. The author's survey by examination and questioning of ninety per cent of the population did, in fact, bring to light an unusually high incidence of some rare congenital disorders, though it also showed the islanders to be remarkably healthy. Not all the author's findings are analysed in this monograph, which deals largely with the incidence of congenital defects of a structural or metabolic type and with a study of eighty-seven consanguineously derived propositi and of a similar number of non-inbred controls.

Having found that the incidence of albinism, deaf mutism, mongolism, Christmas disease, microphthalmia and some other rare defects was remarkably high compared with that in other studies, the author remained unable to explain his results. Inbreeding provided him with part explanation, but it was not enough to account for the high frequency of recessive traits. Migration from the island, genetic drift, selection and mutation were all considered and found inadequate on the basis of current knowledge. The resulting conundrum posed by the author will be a challenge to others interested in population genetics.

Taken all-in-all, the St Helenians seem to be a happy race, though they suffer from asthma to an unusual extent, from ischaemic heart disease in spite of walking habitually, and from hallux valgus. Only in the case of the latter can the disease be clearly attributed to the environment—namely to wearing shoes.

CHARLES STUART-HARRIS

GENETIC COMPLEMENTATION

Molecular Biology and the Origin of Species

Heterosis, Protein Polymorphism and Animal Breeding. By Clyde Manwell and C. M. Ann Baker. (Biology Series.) Pp. xiv + 394. (Sidgwick and Jackson: London, April 1970.) 80s boards; 25s paper.

This book has grown out of the authors' researches into the protein polymorphisms of marine organisms and of poultry, which are here set in the context of related studies and organized around two central concepts; the origin of species, and the use of hybrid vigour in animal and plant breeding. (For good measure, there is also a half-topic, so to speak, on pollution as an evolutionary force.) Although this means that we get two books for the price of one, we have to pay for it: parts of the book are written for breeders, whereas other parts have molecular biologists in mind; and this ambiguity of purpose, and an involved style, make the text difficult to read. Nevertheless, because about 1,000 papers are reviewed, there is much of interest to be gleaned; so it is unfortunate that the index is not organized to this end.

The business of the book is heterosis (hybrid vigour), defined as positive when the hybrid is superior to both parents (following East and Jones), or negative when it is inferior (following Stern, but inverting East and Jones). It is argued that the molecular basis of both is "complementation, either between different polypeptide chains in a protein with a quaternary structure, or between functionally related proteins in a metabolic pathway". These very different possibilities are often confused, and much of the text is given over to explaining the physical chemistry of protein structure and the molecular basis of complementation. The literature on the proteins of heterozygotes is thoroughly reviewed, but the arrangement of the chapters leads to some repetition, and the relevance of a point often becomes clear only in the later chapters when the occurrence of hybrid proteins is related to heterosis. Theory and results are then pushed to their limits (sometimes with the aid of incorrectly computed statistical tests), to "prove" the correlation between complementation and hybrid vigour, positive or negative. After all this it comes almost as a surprise to find that the authors view the successes of hybrid breeding with some scepticism. Their criticisms are not new, but it is useful to have them brought together in one place. The breeder may be forgiven, however, if he is similarly sceptical about their prescriptions for the future: "quantitative genetics for animal breeding will combine not only the mathematics of populations and selection but also the mathematics of allosteric effects and reaction rates". He will remember, if he is a poultry breeder, that blood group polymorphisms were once recommended as sure guides to breeding success. But not any more.

The tabulations in this book suggest that "negative" heterosis is common, and this has to be explained. At least in some instances, the reason is that the samples are too small to give reliable estimates of the deficit of heterozygotes found in a population (for example Table 12), and the reader should be reminded that Raymond Pearl laid down strict rules for the proof of selection of particular genotypes, which are not usually satisfied by the data presented. Of course, selective hybrid elimination is well known, but I take it that the authors mean more than this when they cite "negative heterosis as the internal driving force for speciation", namely, heterozygote disadvantage deriving from particular complementing genes. The case for this concept is not rigorously proven.

Heterosis is usually taken to mean the greater vigour of hybrids in terms of growth, survival and fertility. All of these characters are controlled by many genes, and explanations of this vigour which rest on the characteristics of heterozygous alleles (overdominance), or of complementing alleles, are likely to be suspect. There are

no good examples of overdominance, and there is nothing very new about inter-allelic complementation. None the less, the reader who perseveres with this book will find it useful to have an old problem looked at from a new point of view. But it will be hard work for breeders. The book is well produced, and the paperback edition is remarkably inexpensive.

JAMES H. SANG

BONE PHYSIOLOGY

The Physiology of Bone

By Janet M. Vaughan. Pp. xix + 325. (Clarendon Press: Oxford; Oxford University Press: London, April 1970.) 100s boards; 50s paper.

SINGLE author textbooks become rarer and rarer, but, as they do, their merits become more obvious. One author cannot be an expert in everything. Treatment in great depth becomes easier when twenty authors divide up a small field into twenty even smaller parcels, but the gain in depth is often offset by overlapping or even contradiction between different contributors and a patchwork effect which detracts from the overall value and readability of the work.

This is not to decry the value of multiple author books but simply to emphasize that works by single authors, when well done, are characterized by an internal harmony and rhythm which enhance their value and the pleasure that can be derived from them. This volume is an excellent example of the species. Written with the judgment that comes from honourable retirement, produced so fast that it is uniformly up to date, and illustrated with exceptional clarity, it will be well received by workers in the calcium field and will no doubt run to as many editions as the author has the strength to produce.

Bone physiology is not a well defined field and this first book on the subject is therefore a trend-setter. The decision as to what should or should not be included under this title must be an arbitrary one, the choice influenced by the inclinations of the writer. Dame Janet Vaughan has chosen well. The principal chapters cover the structure, function and blood supply of bone and cartilage and their constituent cells, as well as the chemistry of the mineralized and non-mineralized components and the mechanism of calcification. She then proceeds to the flow of the relevant ions through the body fluids and in and out of bone, and discusses the hormones and other factors which influence these homeostatic processes. She dwells lightly on pathological conditions, reviews the relevant vitamins and concludes with an excellent chapter on the enzymes in bone.

It is a competent performance. The break-up into sections, sub-sections and sub-sub-sections makes for easy reference and the illustrations are not only profuse and excellently reproduced but chosen with admirable skill and judgment. The bibliography is solid without being extravagant or pretentious and creates the definite impression that only useful papers actually consulted by the author have been included.

This does not mean that the internal balance of the work cannot be questioned. Nutritionists might well argue that nutritional aspects of bone metabolism are glossed over and that such an important and relevant organ as the kidney receives rather short shrift. In reply the author has only to point to the title of the work which indicates clearly both its scope and its limitations. Bone is the focus of the work and occupies the author's principal attention throughout. The kidney and the gut, though interesting, are kept firmly in their respective places and those of us whose particular interests lie in this direction are not entitled to complain.

In brief, then, here is a good review of a difficult subject which will serve as a reference work for those already in the field and an excellent introduction for those who have not yet moved into it.

B. E. C. NORDIN

AMERICAN ECOLOGY, 1968

Challenge for Survival

Land, Air and Water for Man in Megalopolis. Edited by Pierre Dansereau, with the assistance of Virginia Weadock. Pp. xii + 235. (Columbia University Press: New York and London, April 1970.) 72s; \$7.95.

Books composed from the papers and discussions of conferences carry built-in handicaps, which only the best of them manage to surmount. This one results from a symposium in April 1968 organized by that distinguished and far-seeing ecologist Pierre Dansereau, at that time senior curator in ecology at the New York Botanical Garden which presented the project with the backing of the National Science Foundation.

It is interesting in European Conservation Year to have this further testimony to the convergence of advanced thinking on ecology applied to the total environment in North America with the lines which have been simultaneously developed on the European side of the Atlantic. The style, both in a wider and a strict sense, is refreshingly different, but the material reviewed and the conclusions reached are parallel and similar even where they are not identical. Dansereau's own scholarly essay goes well beyond its title "Megalopolis: Resources and Prospect". Other contributions, such as Frank E. Egler's "Ecology and Management of the Rural and the Suburban Landscape", follow the line of blending a provocative essay-type review with a somewhat sketchy presentation of fact and a number of practical criticisms and proposals.

Helmut E. Landsberg's "Metropolitan Air Layers and Pollution", George M. Woodwell's "Radioactivity and Fallout: the Model Pollution", and David M. Gates's "Relationship between Plants and Atmosphere", help to provide a slightly more solid raft. Several of the papers have already appeared elsewhere in slightly different form. References to literature are on the light side and there is no index.

Challenge for Survival is to be recommended therefore simply as a stimulating series of loosely connected essays by a group of leading American ecologists and others who have something constructive and coherent to say on the present state of ecology and its immense implications for public affairs. Although, as one of the contributors says, we may in a sense have heard it all before, this is one of the best and most succinct statements, in spite of its obvious unevenness, and it will help as a corrective on some of the aspects which European work tends to neglect.

Americans are good at this sort of thing but, as several of the writers remind us, ecology in the United States has been very backward in rising to its social responsibilities. To a European, however, it is somewhat baffling to see so little result in practice to show for so much lively and persuasive illumination as the American record incessantly adds to itself.

E. M. NICHOLSON

FISH DISEASES

Principal Diseases of Marine Fish and Shellfish

By Carl J. Sindermann. Pp. x + 369. (Academic Press: London and New York, April 1970.) 163s.

INFORMATION about diseases in marine animals is widely distributed through the world literature. Dr Sindermann's book provides an excellent summary of existing knowledge. It is an expansion of two earlier reviews^{1,2}.

The introduction forms the first chapter. Nine hypotheses are given about the role of diseases in the marine environment. Chapter two gives an account of the diseases of marine fishes. A short preliminary section indicates how access to the literature is achieved, followed by sections on microbial diseases, including viruses, bacteria, fungi and Protozoa, diseases caused by helminths and parasitic Crustacea, and, finally, genetic and environmentally

induced abnormalities. The diseases of commercially valuable food fishes are emphasized throughout. Each section is divided into appropriate subsections giving a concise account of the range of diseases. A complete review is not attempted; enough information is provided to enable the reader to appreciate the problems involved and to gain entry to the original literature. Drawings or photographs illustrate representative disease symptoms or organisms. Twenty-seven pages of references conclude the chapter.

The same style is followed in the third chapter on the diseases of shellfish. Mollusca and Crustacea are treated separately. The mollusc section is divided into bivalves, gastropods and cephalopods. The section on bivalves is the most extensive; oysters, mussels, clams, scallops and pearl oysters each get individual consideration. Because of the use of these narrower topics, the account is more comprehensive than that given in the chapter on fish diseases. The Crustacea are divided into three subdivisions, crabs, lobsters and shrimps. Fewer illustrations are provided than in the previous chapter. There are twenty-one pages of references for molluscs and eleven for crustaceans.

A basic review of the range of disease organisms is thus provided in chapters two and three. The treatment is thorough, but not exhaustive. It is not intended to provide a means of identification of disease symptoms or organisms, but it does provide a good introduction to the areas of study.

The remaining chapters deal with six topics of importance in relation to disease in the marine environment. For many readers they will be the most interesting part of the book. The following topics are considered: mortalities of marine animals, with emphasis on the role of disease in North American clupeoid fishes and shellfish, disease and parasite problems in marine aquaria and cultivated marine populations, vertebrate and invertebrate defence mechanisms, the relationship of human diseases to diseases of marine animals, and an assessment of the role of disease in the marine environment. Finally, there is a brief discussion of problems of future studies of diseases in the marine environment. References are given at the end of all chapters. There are author and subject indexes.

JAMES C. CHUBB

¹ Sindermann, C. J., *Adv. Mar. Biol.*, **4**, 1 (1966).

² Sindermann, C. J., and Rosenfield, A., *Fishery Bull. US Fish Wildl. Serv.*, **66**, 335 (1967).

PROTECTORS AND SENSITIZERS

Radiation Protection and Sensitization

Edited by Harold Moroson and Marcello Quintiliani. (Proceedings of the Second International Symposium of Radiosensitizing and Radioprotective Drugs, Rome, May 1969.) Pp. xvi + 522. (Taylor and Francis: London, March 1970.) 160s.

THE design of this symposium was good; a discussion of both radiation protection and sensitization together at different levels of organization. Unfortunately, no account of the discussion after the various papers appears in the published proceedings, which consist of five review articles and sixty-eight shorter contributions, some of which are good and some of which are slight.

It is clear that while work on molecular and single cell systems is devoted more to sensitization than to protection, the emphasis in multicellular studies is still on protection. Adams reviews some model radiation chemical and cellular systems in a clear and easily read account, dealing generally with both mechanisms, as does Alexander and his colleagues' review of DNA repair in two different cell systems, mouse lymphoma and *Micrococcus radiodurans*. The section on single cell systems contains a good account from Block, Mulligan, Weidner and Doherty of change from protective to sensitizing effects with substitution in a series of chelat-

ing agents, Sinclair's interesting studies of the opposed effects of hydroxyurea and cysteamine in different phases of the cell cycle, and evidence from Duplan and Fuhrer that mitomycin C can be considered to be a true radiosensitizer of colony forming mouse bone marrow cells.

With multicellular systems, protection studies are dominant. Pihl and Sanner review the protective effects of thiols and disulphides in mammals, and Semenov discusses the non-sulphur containing protective agents. This review is much shorter than the other four and would have been more valuable had it been expanded. Twelve out of seventeen papers on multicellular systems describe protective effects, and the quality of these contributions is uneven, although Sztanyik and Várterész make an interesting attempt to analyse the synergism between the protective actions of 2- β -aminoethylisothiourea (AET) and 5-methoxytryptamine in mice. The group of contributed papers on the pharmacology of protective and sensitizing agents is disappointing.

Kaplan gives an admirable and up to date review of the laboratory and clinical use of the halogenated pyrimidine base radiosensitizers, and the contributed papers on clinical uses contain an excellent account by Hoshino, Nagai, Sato, Sano and Watari of the treatment of malignant gliomas by the combined use of antimetabolites, 5-bromo-deoxyuridine (BUdR) and fractionated radiotherapy.

The book is handsomely produced, as its price would suggest. As all papers begin on the right hand page, there are some empty pages (10 per cent, in fact, are blank or contain only a few lines of references), so that one regrets the omission of any discussion even more. Most of the papers are clearly written, but there is an onus on the editors of volumes containing contributions from those writing in a language not their own to prevent phrases such as "rephlectory vasomotorial disturbances" appearing in print. The book should be useful to those actively working in this field, but the mixed quality of some of its contents make it a less straightforward choice for a general radiobiology library.

JENNIFER SHEWELL

SEAMEC AND ITS JOURNAL

The South-East Asian Journal of Tropical Medicine and Public Health

Vol. 1, No. 1. Quarterly. (Central Coordinating Board for Tropical Medicine and Public Health Project by SEAMEC: 420/6 Rajvithi Road, Bangkok, Thailand, March 1970.) \$6.00 per volume.

THE present trend for closer cooperation between countries belonging to a more or less definable geographical, socio-economic or cultural region is particularly evident in south-east Asia. In that part of the world seven countries (Indonesia, Laos, Malaysia, Philippines, Singapore, South Vietnam and Thailand) have formed a south-east Asian ministers of education organization (SEAMEC) which has its permanent council in Bangkok under the direction of its secretary-general Professor Chamlong Harinasuta.

In 1966 this body set up a regional scheme of medical and public health research and training through the co-operative effort of existing national centres. The funds are provided jointly by the countries themselves and by the United States government. Several conferences, seminars, postgraduate courses and an exchange of scientists have been organized under this scheme. It is believed that this training programme will be more appropriate and less expensive than sending the nationals of the countries involved to institutes and schools in the western world. This should also prevent the brain drain from the developing world to the advanced countries.

Among its many services to the community, SEAMEC has now brought out this new journal on tropical medicine and public health. It contains a foreword by Professor B. G. Macgrath, a statement of the *raison d'être* of the

journal by Professor A. A. Sandosham, its chief editor, and an outline of the SEAMEC's aims, ways and means by Professor Ch. Harinasuta.

The scientific content of the journal is varied, and the subjects range from parasitology and entomology to planning of undergraduate education in south-east Asia. Among the eighteen scientific papers of this issue, many are of particular interest. Parasitology *sensu lato* opens with the recent finding of trypanosomes in Malaysian macaques, their transmission by a local *Triatoma* and consideration of their infectivity to man. Other subjects are: Brugian filariasis in Thailand, attempts to immunize cats with irradiated larvae of *Brugia malayi*, leptospirosis in West Malaysia, and immuno-diagnosis of parasitic infections. Other relevant topics comprise the attempt at control of *Schistosoma spindale*, research on molluscan hosts of *Angiostrongylus cantonensis*, experimental double and triple infection of snails with larval trematodes, fascioliasis in Thailand, technique of permanent mounting of eggs and larvae of helminths, action of diethylearbamazine on rats, and a malaria survey on Bougainville Island. Entomology covers the bionomics of *Anopheles* in the western Pacific and fenthion resistance in *Culex fatigans*. A study on haemoglobin B₂ in West Malaysia, an investigation of nutrition and environment in Iban longhouses in Sarawak, discussion of the use of community health projects for undergraduate medical education and an account of a series of laboratory demonstrations complete this first issue.

One can only admire the initiative, the intelligence and the scientific vigour of the south-east Asian research community gathered under the flag of SEAMEC, and the new journal deserves our best wishes and our active support.

L. J. BRUCE-CHWATT

CRUSTAL DISPLACEMENT

Time and Place in Orogeny

Edited by P. E. Kent, G. E. Satterthwaite and A. M. Spencer. (Special publication No. 3.) Pp. viii + 311. (Geological Society of London: London, 1969.) 150s.

AFTER a century and a half of intensive geological research we are scarcely nearer to a clear assessment of the nature, amount and rate of crustal displacement involved in the evolution of continental areas; this at a time when our knowledge of the displacements within the oceanic crust is progressing by leaps and bounds. It was with the aim of rectifying this situation that the Geological Society of London, together with the Yorkshire Geological Society, invited sixteen authors to present papers at a symposium at Durham in January 1968, entitled "Displacement within Continents". This volume, the first of a three-fold presentation leading to a synthesis of continental displacement within the later Mesozoic and Tertiary orogenic belts, presents the sixteen symposium papers in an attempt to review the problems involved and to define the parameters for measuring displacement.

The volume is divided into three sections. The first of these consists of five contributions dealing with the structural analysis of orogenic belts. These papers deal with aspects ranging from the intractable problem of interpreting deep-seated movements from surface structures to improved methods of constructing realistic horizontal sections through regions of complex structure. An important paper dealing with the measurement of strain and displacement difference from a theoretical standpoint suggests practical methods for determining crustal shortening over the entire width of an orogenic belt. The role of palaeomagnetic studies is highlighted in the final paper of this section.

The second section contains three papers concerned with the correlation and dating of orogenic events. A natural bias towards radiometric dating is admirably foiled by a

definitive and constructive account of the science of geological correlation.

The third and final section contains six papers dealing with individual aspects of the study of orogenic belts. The need for an independent tectonic terminology for orogenic belts is emphasized in a contribution which surveys the variety in orogenic belts. In another the rate of displacement, both vertical and horizontal, is considered. Three papers stressing the anomalous nature of the deeper crust within orogenic belts, both in physicochemical characteristics and in relation to its excessive thickness, suggest possible geophysical methods for determining meaningful models of the displacement within continents.

It is pleasing to see a section devoted to discussion, both that following the reading of the papers at the symposium and also that occurring later as written contributions. This section, which serves as a detailed criticism of individual papers, disappoints only in the paucity of points raised.

In such a collection of papers from a range of authors it is to be expected that not all the contributions rise to the level of the best. *Time and Place in Orogeny* is, however, a volume that few structural geologists and geotectonists can afford to be without, and all will anticipate with considerable interest the publication of the second volume of the trilogy: "Data for Orogenic Studies", the aims and organization of which are laid out at the end of this volume.

R. A. GAYER

ARCH PIONEERS

A Span of Bridges

An Illustrated History. By H. J. Hopkins. Pp. 288. (David and Charles: Newton Abbot, June 1970.) 70s.

THE building of bridges is still an exciting branch of engineering, because the challenges it presents never become stereotyped. No two bridges are exactly the same; each time the problems of wind and water, the lie of the land and the levels of the approaches have to be tackled afresh. Thus the Forth and Severn road bridges, finished within two years of one another, solve apparently identical problems in subtly different ways. The bridge designer also needs an eye for proportion and scale; the finished structure must not only do its job, but must be seen to be doing it. Form and function are, of course, closely related, and there are very few great bridges which do not "look" right, even to the untrained eye.

Professor Hopkins has written an excellent history of the art, the craft and finally the profession of building bridges. It is a story well provided with interesting characters, starting with Vitruvius, whose writings of the first century BC establish beyond doubt that the Romans fully understood the principle of the arch. Just before the French Revolution, Jean-Rodolphe Perronet brought this same principle to perfection with his daringly low arches and slim piers at the Pont de la Concorde in Paris. The doubters, of course, predicted instant collapse as soon as the centre supports were removed, but the bridge stands today.

After Perronet came the iron bridges of the nineteenth century—some were triumphs, like Robert Stephenson's recently damaged Britannia Bridge across the Menai Straits, or Telford's Mythe Bridge at Tewkesbury, and some were well remembered disasters, like the "wondrous bridge across the silvery Tay", as McGonigall just had time to call it in the brief space between completion and collapse. Today the suspension bridge, refined to remove dangerous oscillation and twisting, reigns supreme for large structures. Pre-stressed concrete has taken over for the smaller bridges, and it, too, has its masterpieces. Hopkins picks out for particular praise Robert Maillart's Schwandbach and Salgina bridges, both unbelievably light and elegant structures. He also has a good word for Waterloo Bridge across the Thames, whose designers had

to cope not only with nature and the Blitz, but also Sir Giles Gilbert Scott, an eminent architect thrust on them. Londoners have reason to be grateful for their patience and determination, for one has to go upstream as far as I. K. Brunel's Maidenhead railway bridge for a Thames crossing of comparable distinction.

This book will be enjoyed by sixth-formers, undergraduates and armchair engineers everywhere. It is profusely illustrated and admirably written. My only complaints are the uneven quality of the printing and rather haphazard layout; but these are quibbles.

NIGEL HAWKES

DEFECTS IN CRYSTALS

Crystallography and Crystal Defects

By A. Kelly and G. W. Groves. Pp. xi + 428. (Longman: London, May 1970.) 100s.

THIS lucid and interesting book sets itself the somewhat ambitious task of combining the elements of crystallography, stereographic projection, tensor algebra and elasticity theory and of using these in a thorough study of crystal defects. Moreover, the book is aimed at students as widely separated as postgraduates and first year undergraduates. What is important is that it succeeds to a quite remarkable extent. The introduction takes up the first five chapters (part one plus chapter five), and is concisely and accurately presented. Then follows the development with chapters on glide, dislocations, point defects, twinning, martensitic transformation and crystal boundaries. It is necessarily selective, stressing consistently the crystallographic nature and importance of defects, and is refreshingly clear and direct. I liked particularly the discussion of Peierls force and grain boundaries and the chapters on twinning and martensitic transformation. I was disappointed that the discussion of dislocation core structure was not more closely related to crystallography. The book is particularly complete on subjects which the authors have pioneered (for example, independent slip systems).

This is essentially a text, not a reference book. It will certainly be of interest to postgraduates, though I feel that some undergraduates will find its style a little too brief and lacking in development. The book is excellently produced, has useful problems at the end of each chapter with answers at the back, and several valuable appendices. Wherever possible it avoids mathematical detail. In fact, my only criticism of substance is that it has tried to do too much. Our understanding of the importance of the crystallographic nature of defects in crystals is too incomplete at present to allow a full discussion. In many cases where the importance of crystallography is established, a proper discussion would involve prohibitive mathematical complexity, for example, anisotropic elasticity which is only hinted at in this text. We shall have to wait for an authoritative treatment of the subject. J. W. STEEDS

PARTICLE STATES

Elementary Particle Theory

By A. D. Martin and T. D. Spearman. Pp. xiii + 527. (North Holland: Amsterdam, 1970.) Hfl. 83; 193s; \$22.

THE most useful theoretical concept for the analysis of the dynamics of strong interactions between elementary particles which is available to us at present is the scattering matrix (or S-matrix) element, which may be regarded as the quantum mechanical probability amplitude for scattering from an initial to a final state. The S-matrix element is a function of the external parameters in the process such as energy and angle of scattering, and the hope is that this

functional dependence may be determined by the addition of general postulates such as relativistic invariance and conservation of probability. The most powerful additional hypothesis seems to be one about the singularities of the S-matrix elements considered as analytic functions of their variables, which is suggested by the examination of certain models. When supplemented by the Regge pole description of scattering processes at high energies, these assumptions lead, at the least, to relations between S-matrix elements which can be tested against experiment and may furnish the basis of a complete dynamical theory.

The purpose of this book is to give a development of these topics in the detail suitable for the graduate student intending to do theoretical research in the field. A particular feature is the treatment of the kinematics of the one, two, or more, free particle states in terms of which the S-matrix elements are defined. Because the concepts of quantum field theory do not enter into the scheme outlined earlier, the description of free relativistic particles using field equations has been omitted in favour of an equivalent one, which is based on the notion that a complete set of states for a free elementary particle should form the basis for an irreducible representation of the inhomogeneous Lorentz group. The necessary group representation theory is developed without assuming previous knowledge of the subject in the first half of the book, although, occasionally, objects such as the tensor product symbol have slipped in without a precise definition. Isospin and the discrete symmetries P, C, and T are discussed and applied, but not SU(3).

In the second half of the book, the analytic properties of two particle scattering amplitudes and partial wave amplitudes are discussed. Building on the material of the first half, a detailed treatment of the analyticity and crossing properties of helicity amplitudes for arbitrary spin is given, following the approach of Trueman. An excellent final chapter on Regge poles motivates their introduction and brings the reader to within sight of recent activity on the Veneziano representation and its generalizations. The absence of any field theory means assumptions about analyticity, crossing symmetry and kinematic singularities are not motivated by an examination of Feynman graphs. The beginner must look elsewhere for these.

Sets of useful exercises with hints for their solution are provided at the end of each chapter. Students who master this material will be well equipped to undertake research in this field. The presentation in a uniform notation of the topics covered should also make the book a convenient reference for practising theorists. B. R. POLLARD

REACTOR KINETICS

Space-time Nuclear Reactor Kinetics

By Weston M. Stacey, jun. (Nuclear Science and Technology: a Series of Monographs and Textbooks, Vol. 5.) Pp. vii + 186. (Academic Press: New York and London, December 1969.) 121s.

DURING recent years there has been a considerable amount of technical literature on the subject of spatial reactor kinetics, due largely to the inability of the older point kinetic models to describe the transient behaviour of large power reactors, where spatial effects are of considerable importance. This book provides a useful text that bridges the gaps between the older point kinetic models and the more modern spatial approaches.

The reader is expected to have had a basic grounding in reactor kinetics and physics. A basic knowledge of matrix arrays would be an advantage.

The text begins with the now familiar time dependent group diffusion equations and deals with the spatial finite difference approximations. Modal expansion approximations utilizing matrix methods are evaluated, proceeding

through natural modes, Green's function and synthesis modes. Nodal approximations are then considered in detail. The various methods are compared and their respective advantages and disadvantages are discussed for particular applications.

Numerical integration methods are covered in some detail; these will provide the reader with a good foundation to build on, because many subsequent variations lie in the hands of the user. A good discussion is given of stability truncation errors and computation time.

Variational synthesis methods are introduced to allow the general space-kinetics problem to be approached systematically. This leads to multichannel space-time synthesis which makes more efficient use of a given set of expansion functions, a good example being given to illustrate this technique.

Up to this point the book deals with computational methods for solving the spatially dependent kinetic equations and provides the reader with a good broad introduction. The neutron process is stochastic in nature and chapter 4 covers the use of a forward stochastic model which leads to the use of correlation functions. A theory for backwards stochastic model is given and the Langevin technique is discussed in some detail. This chapter is mathematical, and some knowledge of stochastic functions would assist the reader.

There is a good study of xenon spatial oscillations using linear and non-linear techniques. Control induced xenon spatial oscillations are introduced and their control is illustrated. The last two chapters serve to present a basic theory with which to treat the stability and control of spatial reactor models.

To summarize, the book gives an excellent, though mathematical, approach to the subject of space and time dependent reactor kinetics. It contains numerous useful references which will enable the reader to probe more deeply into the subject and, in general, it fulfils its object of bridging the gap between new and old texts.

ALAN JEBB

KINETIC THEORY OF GASES

The Mathematical Theory of Non-Uniform Gases

An Account of the Kinetic Theory of Viscosity, Thermal Conduction and Diffusion in Gases. By Sydney Chapman and T. G. Cowling. Third edition prepared with the co-operation of D. Burnett. Pp. xxiv + 423. (Cambridge University Press: London, May 1970.) 100s; \$16.

THE first edition of this book appeared in 1939 and immediately established itself as an authoritative work on the kinetic theory of gases. This is the third edition of the book in which the already concise and elegant presentation of the equations and text has been improved by printing in a modern format and adopting a new layout of the text. The work has been the basis for the development of the subject matter since the initial publication of the book. The authors, renowned for their contributions in their respective fields, have been aided by Professor D. Burnett in incorporating the most recent developments in the theory.

The chapter dealing with molecular models with internal energy has been completely rewritten to take into account recent advances. The theory of multiple gas mixtures is dealt with for the first time and warrants a chapter of its own. The discussion of the electromagnetic phenomena of gases has been greatly extended to include major contributions that have taken place in this rapidly changing field since 1952, and includes the Fokker-Planck approach to the Boltzmann equation and the subject of collisionless plasmas. The authors have deemed it necessary to omit some of the subject matter that was in the previous edition, but nothing of substance has been left out.

The revision of this substantial work (essential for all research workers in this field) is wholly welcome. Furthermore, it can be thoroughly recommended for those without a copy of the previous edition in their possession. It is, however, hard to justify bringing one's personal library up to date in view of the small number of changes that have been made to the text.

A. L. T. POWELL

ALGEBRAIC TOPOLOGY

The Topology of Classical Groups and Related Topics

By S. Y. Hussein. Notes on Mathematics and its Applications. Pp. viii+128. (Gordon and Breach: London and New York, February 1970.) 95s; \$11.40.

THIS book is based on notes of lectures first given in 1963 to University of Wisconsin graduate students who had previously undertaken a basic course in algebraic topology. Although the material has been polished up in the writing, the overall impression is still one of lecture notes rather than of a finished work. For example, the book has no index. Thus forewarned, the reader will need pen and paper as a constant companion as he journeys through the book.

In spite of or perhaps because of this, the author covers a lot of ground. In the first chapter fibrations are treated together with universal fibrations and various classification theorems are given. After the start of the second chapter, the author defines the concept of an algebra in a universal fashion (in fact, the whole book is very modern in style). Having done this he is then free to dualize and define a co-algebra and a Hopf algebra. The rest of the chapter is devoted to calculating the homology and cohomology of the classical groups. In chapter three the author considers the homology and cohomology of the classifying spaces and loop spaces of the classical Lie groups. This naturally leads on to Bott periodicity and, finally, in chapter four, to K-theory.

ROGER FENN

MODERN CATALYSIS

Catalysis of Gas Reactions by Metals

By A. J. B. Robertson. Pp. xi+182. (Logos, in association with Elek Books: London, June 1970.) 70s.

THE development of comparatively straightforward techniques for reducing the pressure in a laboratory-sized apparatus to the ultra-high vacuum region has had a stimulating effect on the study of the reactions of metal surfaces with simple gases. The reason for this is that by producing background pressures in this region it has become possible to study a chosen reaction in the complete confidence that the residual gas does not make a significant contribution to the observations. The reliability of the measurements has been of great importance in view of the sometimes unexpected results which have been reported. Dr Robertson is well known for his contributions in this area of research and in his book he has chosen to give prominence to some reactions which have been studied under ultra-high vacuum conditions.

In the first two chapters, which constitute about one third of the book, the evolution of ideas about catalysis is traced from the contributions of Davy and Faraday in the early nineteenth century to modern deductions about the highly selective reactivity of the individual planes present on a surface. Almost all of this discussion and of the contents of the next chapter, which gives a brief account of ultra-high vacuum techniques, should be readily comprehensible by sixth-formers. Beyond this point the book gets noticeably harder, though the liberal use of easily understood diagrams and a lucid exposition should allow much of it to be read with profit by this group and by undergraduates in their first year.

The second half of the book describes the distinctive aspects of the contemporary approach to catalysis. Modern techniques for cleaning and studying metal surfaces are described systematically, though briefly. No doubt deliberately, the author refrains from much comment on the relative importance and contribution of the various techniques he describes. This is a pity, because comment from this authoritative source could have been helpful to the newcomer to the field.

In discussing the theoretical interpretation of the experimental results the most detailed treatment is given to the approach by way of transition state theory. A sensible balance is maintained in this discussion between the generalized treatment of fundamental ideas and the detailed interpretation of particular experiments.

This is a book which I enjoyed reading and which I shall recommend to undergraduates for their first encounter with the modern approach to catalysis. It is, unfortunately, presented at a price at which they cannot reasonably be expected to buy their own copy. R. P. H. GASSER

PERSONAL RELATIVITY

Cosmology

By Jean Charon. Translated from the French by Patrick Moore. (World University Library.) Pp. 256. (Weidenfeld and Nicolson: London, July 1970.) 35s boards; 18s paper.

THIS would be an admirable book if it were not for one chapter in which the author presents a personal view of the implications of the theory of relativity, drawing conclusions which differ markedly from the accepted point of view, and which are founded on an extremely uncertain basis. The first eleven chapters give an informative, readable account of the development of man's ideas about the universe, and if the book were concluded after them I would be glad to recommend it to the many people who are interested in cosmology and astronomy but have no mathematical background.

On the cover of the book, a summary states that "The last chapters discuss the possible meanings of space, time, distance... Some of the author's ideas are new and exciting, and are explained in a way which will appeal to both the beginner and the specialist". This latter claim is far from the truth, because although Charon's ideas will certainly appeal to the beginner who knows no better, the specialist is likely to be infuriated by his glib presentation of a biased and unreliable interpretation of the implications of relativity theory as accepted facts. Any specialist reading this review will doubtless have guessed that a large part of the misconceived chapter to which I have referred hangs on an erroneous interpretation of the so-called "twin paradox" of relativistic time dilation. This is certainly not the place to present a detailed refutation of Charon's reasoning—the specialist is already familiar with this (because it has been known for 50 years that there is no paradoxical situation at all), and the non-specialist would be better referred to a standard introductory text such as *Einstein's Theory of Relativity* by Max Born (Dover, 1962).

Because on balance the harm which might be done by the latter part of this book would probably outweigh any benefit derived from the earlier part, I would not recommend it to anybody completely new to the subject, but for those interested in the history of astronomy, and who have either sufficient knowledge of relativity to be unmoved by chapter 12 or sufficient faith in me to ignore it, I would offer a cautious recommendation, although the same historical ground has been covered by other authors in books which do not misinterpret Einstein's theories.

JOHN GRIBBIN

Correspondence

An Unidentified VLF Station

SIR,—During a search of the VLF portion of the radio spectrum for transmitting stations which might serve as sources of “whistler-mode” signals, we observed a transmission on 14.9 kHz which presented some puzzling propagation effects.

Its origin is unknown, and we have been unable to identify the operating agency or the location of the transmitter itself.

We have plotted the diurnal phase variations of the transmitted frequency for about six months, and, by correlating this information with the sunrise and sunset terminators which produce the major phase variations, we have determined that the signals arrive from the direction of the Gulf of Alaska (about 30° true). The great circle distance from Lower Hutt is indefinite, except that it is more than 8,000 kilometres.

Variations of signal strength suggest that at least a portion of the path is subject to PCA events. The station cannot be in Europe or Africa, or its signals would be received along the short great circle path, which can be readily identified by means of the sunrise–sunset phenomena.

There is some evidence that the frequency is controlled by an oscillator which is corrected daily by reference to an atomic standard.

The station is using one of a group of six frequencies used by an unidentified agency during 1969 to transmit a repetitive sequence of short dashes, similar to the well known Omega format, except that the pattern consisted of six intervals over 3.6 seconds, as against the Omega pattern of eight intervals over ten seconds.

No information on the transmission described, or its “navigational” predecessors, which ceased transmission in January 1970, has been noted in the technical or scientific press, and any information on the existing transmissions on 14.9 kHz known to other scientific workers in this field would be welcomed by this laboratory.

Yours faithfully,

M. C. PROBINE

Physics and Engineering Laboratory,
DSIR,
Private Bag,
Lower Hutt,
New Zealand.

Topology and Metallurgy

SIR,—In a recent publication¹, L. Barrett and C. Yust review the applications of topology to metallurgy. From it, it is clear that metallurgists have been laggard in using topology: after a pioneering paper by C. S. Smith in 1951 (ref. 2) on the analysis of grain shapes in a polycrystal, only some 15 to 20 of the papers which have been published up to now on this problem, on the sintering process and on the study of interfaces in a two-phase material, have applied topological theorems.

This lack of enthusiasm is really surprising because, by its very nature, topology seems to be a very appropriate tool to deal with metallurgy, a brand of science that proposes the “wary analysis of non-living complexity”³, “a perpetual balancing act between the realistic and the tractable”⁴. As Barrett and Yust put it: “Often mathematics, particularly applied mathematics, is thought of

in terms of analytical formulae yielding quantitative results. (Topology) is of a more qualitative nature, offering newer concepts as the means of describing physical phenomena. . . . These concepts yield a fresh viewpoint and unifying relationships among apparently diverse structures”. In typical metallurgical problems as complex as work-hardening, recrystallization, creep and the like, metallurgists have been so busy—and not very successfully as yet!—looking for analytical formulae that they have overlooked the possibility that perhaps topological relationships were more apt to answer their questions. I do not see any epistemological reason why nature must abhor topology. And in fact I would like to suggest that perhaps nature loves topology, as is shown by what I would call the “topological structure” of one of the most important relationships in physical chemistry, very well known, respected and used by all kinds of metallurgists: Gibbs’s rule of phases.

Gibbs’s rule possesses a striking similarity with Euler’s theorem (which gave rise to the discipline of topology): If we call V the number of vertices, E the number of edges and F the number of faces of a polyhedron, then Euler’s theorem states that $V - E + F = 2$. But if V is now the number of phases, E the number of independent components and F the number of degrees of freedom of a physico-chemical system, Gibbs’s rule states that $V - E + F = 2$.

It can, of course, be said that this similarity is merely an accident, a formal curiosity without any further consequence, and that, besides, Gibbs’s rule is not more than a useful “recipe”, deduced from a system of analytical equations establishing the equilibrium conditions of a system of V phases and E components with F degree of freedom. But the important thing to realize is not how the “recipe” is usually deduced, but its very nature: just as Euler’s theorem makes possible a “geometry without metrics”, Gibbs’s rule permits one to analyse equilibrium without measuring anything. It provides a powerful means of dealing with the many variables of the equilibrium of any complex system. To evaluate how powerful it is, it is enough to try to imagine metallurgy without Gibbs’s phase rule.

Are there many other topological relationships in the realm of metallurgy? According to Barrett and Yust, there are very few, and these do not seem to be as important as Gibbs’s phase rule. This paucity is not strange, because it seems that metallurgists have not been looking very hard for applications of topology to their science.

Yours faithfully,

JORGE A. SABATO

Departamento de Metalurgia,
Comision Nacional de Energia Atomica,
Buenos Aires, Argentina.

¹ Barrett, L., and Yust, C., *Metallography*, **3**, 1 (1970).

² Smith, C. S., *Metal Interfaces* (Amer. Society for Metals, Cleveland, 1952).

³ Smith, C. S., *A historical view of one area of applied sciences: metallurgy*, *Applied Science and Technological Progress* (National Academy of Science, USA, 1967).

⁴ Cahn, R. W., *Discovery*, 41 (July 1965).

Abortion Act in Action

SIR,—Since the publication of my letter on May 16 (*Nature*, **226**, 673; 1970) more recent official figures¹ have shown that abortion deaths in 1969 were in fact a little up on 1967, the last full year under the old law.

In England and Wales, 10 deaths were directly attri-

buted to legal therapeutic abortions in 1969, not including 4 registered under the Act as resulting from legal operations after illegal or spontaneous abortions. In addition, 8 deaths "from other underlying causes after legal abortion operations" were registered under the Act. Of the latter, 4 resulted from conditions (congenital heart disease; cancer of rectum; cerebral haemorrhage; acute myocarditis) which were probably the reasons for, rather than the results of, the abortion operations. But the other 4 (3 pulmonary embolisms; 1 respiratory obstruction while recovering from anaesthetic) seem themselves more or less directly attributable to the operation, in the sense that these deaths probably would not have occurred had the pregnancies not been terminated. So there were certainly 10 deaths, and probably 14, resulting from legal abortions in 1969.

Also "a further 25 deaths were classified as directly due to abortions other than under the Act"² so that the total for all abortions (legal, illegal and spontaneous) was certainly 35 and probably up to 39 in 1969, as compared with 34 for 1967. It is true that 1967 seems to have been a good year, since the Registrar General recorded 53 abortion deaths for 1966, and 50 for the transitional year 1968 (4 months under the old law and 8 under the new); but plainly there is here as yet no good evidence of any

dramatic reduction in mortality resulting from the Abortion Act 1967, as has sometimes been claimed.

Yours faithfully,

C. B. GOODHART

Gonville and Caius College,
Cambridge.

¹ Crossman, R., *Hansard (Commons)*, written answers, col. 560 (May 28, 1970).

² Crossman, R., *Hansard (Commons)*, written answers, col. 272 (March 23, 1970).

Blue Streak's Record

SIR.—I should be interested to know by what criterion you judge Blue Streak to be "unreliable" as the first stage of the Europa launcher (*Nature*, 227, 425; 1970). Ten successful firings out of ten seems to me to deserve a slightly kinder description.

Yours faithfully,

R. H. W. BULLOCK

Ministry of Technology,

Prospect House,

100 New Oxford Street, London WC1.

MR BULLOCK is right. Blue Streak is reliable. But there is nothing to modify the assertion in the leading article that it is otherwise unsuitable as a starting point for a comprehensive European space programme.—Editor, *Nature*.

Obituaries

Professor F. T. von Brücke



FRANZ THEODOR VON BRÜCKE, professor of pharmacology and toxicology at the University of Vienna, died on March 24, 1970, at the age of 62, after a short illness.

Brücke's capability for independent research was apparent from a paper on the epiphyseal cartilage of long bones which he published while he was a medical student. His later development was profoundly influenced by such distinguished teachers as Otto Loewi and H. H. Dale, under whose guidance he gained experience in the pharmacology of the central and peripheral autonomic nervous system, an area of research to which he devoted the greater part of his life. It was therefore no accident that he was drawn to the Vienna school of pharmacologists, pioneered by H. H. Meyer and his successors, E. P. Pick and R. Rössler, whom he followed to the chair in 1948.

As early as 1935, Brücke recognized the antagonism of bulbocapnine to some of the central effects of apomorphine, an interesting observation in view of the now established interrelationship between dopamine, apomorphine and bulbocapnine. This led later to studies on the different

patterns of release of epinephrine from the adrenal medulla after electrical stimulation of the hypothalamus and occlusion of the carotid sinus. An observation of far-reaching theoretical importance was that small amounts of acetylcholine, injected into the skin, elicited a pilomotor response, whereas larger amounts abolished the effect of sympathetic stimulation.

Brücke investigated the effect of denervation of the superior cervical ganglion of the cat as manifested in its sensitivity to adrenaline and acetylcholine and in cholinesterase depletion. Investigations into the innervation of the rabbit cardia led him to postulate the presence of adrenergic fibres in the vagus and cholinergic fibres in the splanchnic nerve. The induction of spasm of the rabbit cardia following severance of the vagus proved to be a simple device for the evaluation of spasmolytic agents. Brücke also studied the influence of acidity and basicity on the cardiac glycosides and described the importance of the binding of digitoxin to protein.

With the increase in his administrative responsibilities as chairman of a large research and teaching department, Brücke's own involvement in laboratory work decreased in later years, but he continued to stimulate research among his students and co-workers. His ability to generate enthusiasm attracted talented young investigators who have since taken up important academic positions in Europe, the USA and Canada. Under Brücke's direction the Vienna school embarked on a broad investigation of dicholinesters of dicarboxylic acids as muscle relaxants which led to the introduction of succinylcholine into anaesthesiology. Another line of inquiry initiated by Brücke was the investigation by electroencephalography of hippocampal activity in the rabbit.

Brücke was recognized as an outstanding authority in matters of public health and in his capacity as an adviser to the pharmaceutical industry. His editorship of the *Wiener Klinische Wochenschrift* won for the journal a high reputation; his communications, both oral and written, sparkled with lucidity and wit. It is no exaggeration to say, too, that his acumen and foresight spared his country the ravages of thalidomide.

Announcements

University News

Dr James A. Dunne has been named project scientist at the Jet Propulsion Laboratory, **California Institute of Technology**, for the 1971 Mariner mission to Venus and Mercury.

Mr Bryan Campbell Clarke, University of Edinburgh, has been appointed to the newly established chair of genetics in the **University of Nottingham**.

Appointments

Mr A. Goodson has been appointed head/space in the **Ministry of Technology** in succession to **Mr R. H. W. Bullock**. Mr Bullock has been appointed deputy secretary.

Dr Harold R. Fletcher has retired as Regius keeper of the **Royal Botanic Garden, Edinburgh**. He is succeeded by **Mr Douglas M. Henderson**, formerly principal scientific officer at the Garden.

Mr A. A. Gray, at present a deputy chairman of the **Wellcome Foundation Limited**, will succeed **Sir Michael Perrin** as chairman when Sir Michael retires at the end of this year.

Sir Harry Melville has been appointed chairman of the **Advisory Council on Research and Development for Fuel and Power**, with effect from October 13, in succession to **Sir Charles Sykes** who is retiring.

At the annual meeting of **Rolls-Royce Limited**, two new board members were announced: **Mr Ian Morrow**, vice-president of the Institute of Chartered Accountants of Scotland, will become executive deputy chairman, with his principal interest in financial control; **Lord Beeching** is to be a non-executive director, representing the interest in Rolls-Royce of the Industrial Reorganization Corporation.

Miscellaneous

Dr Riccardo Giacconi, executive vice president of American Science and Engineering Inc., Cambridge, Massachusetts, has been nominated for the **Röntgen award in astrophysics** by the Physikalisch-Medizinische Gesellschaft, Würzburg. Dr Giacconi is cited for his contributions to X-ray astronomy, including the discovery of X-ray stars and the development of the instrumentation and spacecraft systems required for their observation.

Recent awards announced by the **Academy of Sciences of the USSR** include: the **Vavilov gold medal** for 1970, awarded to **Petr Petrovich Feofilov** for his work on spectroscopy and luminescence of activated crystals; the **Chaplygin Prize for 1970**, awarded to **Samvel Samvelovich Grigoryan** (of the Moscow University Institute of Mechanical Sciences) for his work on mathematical models of explosions in soil and bedrock; and the **Dokuchaev Prize for 1970**, awarded to **Dr Tat'yana Vyacheslavovna Aristovskaya** (of the V. V. Dokuchaev Central Museum of Pedology) for her monograph *Microbiology of Podzolic Soils*, published in 1965.

ERRATUM. In the contents list for the issue of August 1 (*Nature*, 227, xv; 1970), the entry for page 493 should read:

Transmitter release—Characteristics for squid ganglion similar to those for frog neuromuscular junction—**LESTER** (Rockefeller and Woods Hole).

ERRATUM. In the report "Five Hundred Years After" (*Nature*, 227, 541; 1970) the second line should read "the birth of Mikolaj Kopernik, known to the world"

International Meetings

September 2-9, **World, Water and We**, Jönköping (ELMIA AB, Box 6066, 550 06 Jönköping, Sweden).

September 13-16, **Nature Conservation in Norway**, Oslo (Administration for Outdoor Life and Nature Conservation, Ministry of Labour and Municipal Affairs, Kommunaldepartementbat, Oslo Dep, Norway).

September 14-16, **Plastic Pipes**, Southampton (Dr Howard Allen, Department of Civil Engineering, University of Southampton, Southampton C09 5NH).

September 15-18, **Bio-Medical Engineering Exhibition**, London (UTP Exhibitions Ltd, 36-37 Farnival Street, London EC4).

September 21-25, **Plant Protection**, Paris (The Secretariat, Société Française de Phytologie et de Phyto-pharmacie, 57 Boulevard Lannes, 75 Paris 16e, France).

September 29-October 1, **Advances in Polymer Science and Technology**, London (R. H. Craven, Institution of the Rubber Industry, 4 Kensington Palace Gardens, London W8).

September 30-October 2, **Nature Reserves and their Problems**, Bonn-Bad Godesberg (ABN, 53 Bonn-Bad Godesberg 1, Heerstrasse 110, Federal Republic of Germany).

October 13-14, **Reinforced Thermoplastics**, Solihull (The Plastics Institute, 11 Hobart Place, London SW1).

October 13-15, **Petrochemicals and their Raw Materials in Europe: Present Markets and Future Trends**, Budapest (Mr E. Gaal, c/o Chemolimpex, POB 121, Budapest, Hungary).

October 18-24, **Man in the Park Environment**, Canberra (The Grotto, Lower Basildon, Reading, Berkshire).

May 12-14, 1971, **International Powder Technology and Bulk Solids Conference**, Harrogate (Powtech 71, Powder Advisory Centre, 1 Eldon Square, Newcastle upon Tyne NE1 7JG).

May 18-21, 1971, **Electronic Components**, London (Conference Secretary, Electronic Components Board, Mappin House, 4 Winsley Street, London W1N 0DT).

May 31-June 4, 1971, **Biological Aspects of Electrochemistry**, Rome (Professor G. Milazzo, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy).

June 14-18, 1971, **Congress of the European Association of Radiology**, Amsterdam (Congress Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, Holland).

June 27-July 2, 1971, **Improving the Utilization of Energy with Special Reference to Complex Uses**, Bucharest (The Secretary, British National Committee, World Energy Conference, 201-202 Grand Buildings, Trafalgar Square, London WC2).

September 6-11, 1971, **Peaceful Uses of Atomic Energy**, Geneva (International Atomic Energy Agency, Karntner Ring 11, PO Box 590, A-1011 Vienna, Austria).

September 13-17, 1971, **Comparative Leukaemia Research**, Padua (Mrs Patricia Segato, Division of Experimental Oncology, Institute of Pathological Anatomy, Via A. Gabelli 35, 35100 Padova, Italy).

British Diary

Saturday, August 22

Some Zoonoses (Diseases Transmissible from Animal to Man) (two-day symposium) Institute of Medical Laboratory Technology, East Kent Branch, in the Physics Department, University of Kent at Canterbury.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

- Smith Kline and French Foundation. Seventh Annual Report 1969 and Review 1962-69. Pp. 11. (Welwyn Garden City, Herts: Smith Kline and French Foundation, 1970.) [137]
- Ministry of Agriculture, Fisheries and Food. Bulletin 20: Beneficial Insects and Mites. By B. D. Moreton. Pp. vii+118+8 plates. (London: HMSO, 1970.) 17s (85p) net. [137]
- Physics Courses in Colleges of Technology, Polytechnics and Universities, 1970/1971. Pp. 89. (London: The Institute of Physics and the Physical Society, 1970.) [137]
- Science Research Council: Science Board. The Policies and Activities of the Mathematics Committee. Pp. 34. (London: Science Research Council, 1970.) [147]
- Ministry of Technology. Water Pollution Research 1969: The Report of the Water Pollution Research Laboratory Steering Committee with the Report of the Director of Water Pollution Research. Pp. vii+191. (London: HMSO, 1970.) 17s 6d (87p) net. [147]
- Ministry of Agriculture, Fisheries and Food. Department of Agriculture and Fisheries for Scotland. The Brucellosis Incentives Scheme. Pp. 15. (London: HMSO, 1970.) [147]
- Department of Education and Science. On Course Bulletin No. 1: Certificate in Office Studies. Pp. 8. (London: Department of Education and Science, 1970.) [147]
- Report of the Anti-Locust Research Centre for 1969. Pp. iii+49+8 plates. (London: HMSO, 1970. Available from Anti-Locust Research Centre, College House, Wrights Lane, London W8.) [157]
- The Pill: Biochemical Consequences. (Symposium organized by The Association of Clinical Pathologists and The Association of Clinical Biochemists, delivered in London on 27 September 1969.) Edited by Merton Sandler and Barbara Billing. (Supplement to the *Journal of Clinical Pathology*.) (London: British Medical Association, 1970.) 30s; \$4.25. [157]
- Building Research Station. Current Paper 6/70: The Load-Deformation Behaviour of Middle Chalk at Mundford, Norfolk. By J. B. Burland and J. A. Lord. Pp. 10. (Garston, Watford: Building Research Station, 1970.) gratis. [157]
- Natural Gas in the Seventies: The Prospects and Plans of the Gas Industry. Pp. 16. (London: The Gas Council, 1970.) [177]
- British Deer Society Publications. No. 1: Fallow Deer. By Norma and Donald Chapman. Pp. 22. (8 photographs.) No. 2: Muntjac (*Muntiacus* sp.). By Oliver Dansie. Pp. 22 (5 photographs). No. 3: Sika Deer. By M. T. Horwood and E. H. Masters. Pp. 30 (8 photographs). No. 4: Roe Deer. By Peter Delap. Pp. 22 (6 photographs). No. 5: Red Deer. By Peter Delap. Pp. 22 (6 photographs). (Leeds: J. R. Hepper, National Treasurer, British Deer Society, Hepper House, 17a East Parade, 1970.) [177]
- Science Research Council. Daresbury Nuclear Physics Laboratory—Annual Report 1969. Pp. vi+114. (Daresbury, near Warrington: Daresbury Nuclear Physics Laboratory, 1970.) [177]
- King Edward's Hospital Fund for London—Annual Report 1969. Pp. 35. (London: King Edward's Hospital for London, 1970.) [177]

Other Countries

- Museum of Comparative Zoology, Harvard University. Breviora. No. 348: The Relationships of the Skinks Referred to the Genus *Dasia*. By A. E. Greer. Pp. 30. No. 349: The Structural Niche of *Anolis scriptus* on Inagua. By A. L. Laska. Pp. 6. No. 350: *Fimbria* and Its Lucinoid Affinities (Mollusca: Bivalvia). By K. J. Boss. Pp. 16. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1970.) [67]
- US Department of the Interior: Geological Survey. Bulletin 1283: Serpentine and Rodinigte in the Hunting Hill Quarry, Montgomery County, Maryland. By David M. Larrabee. Pp. iv+34+2 plates. Bulletin 1312-1: Distribution of Placer Gold in the Sixes River, Southwestern Oregon—a Preliminary Report. By Sam Boggs, Jr., and Ewart M. Baldwin. Pp. iii+27. \$0.25. Water-Supply Paper 1867: Occurrence of Fresh Water in the Lee Formation in Parts of Elliott, Johnson, Lawrence, Magoffin, and Morgan Counties, Eastern Coal Field Region, Kentucky. By Herbert T. Hopkins. Pp. iv+44+4 plates. Water-Supply Paper 1977: Ground-Water Levels in the United States, 1963-1967. Northeastern States. Pp. v+238. \$1.

Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A12: Fluorometric Procedures for Dye Tracing. By James F. Wilson, Jr. (Book 3 "Applications of Hydraulics".) Pp. viii+31. \$0.35. Professional Paper 626: Geology and Mineral Deposits of the Poncha Springs NE Quadrangle, Chaffee County, Colorado. By Ralph E. Van Alstine. Pp. iv+52. (Washington, DC: Government Printing Office, 1969 and 1970.) [67]

World Health Organization. Monograph Series, No. 59: Fluorides and Human Health. By P. Adler *et al.* Pp. 364. (Geneva: WHO; London: HMSO, 1970.) 30 Sw. francs, 60s; \$10. [67]

Organization for Economic Co-operation and Development. Gaps in Technology—Non-ferrous Metals. Pp. 202. 27 francs; 41s. \$6. Gaps in Technology—Analytical Report. Comparisons between Member Countries in Education, Research and Development, Technological Innovation, International Economic Exchanges. Pp. 300. 36 francs; 55s; \$8. (Paris: OECD; London: HMSO, 1969 and 1970.) [67]

Food and Agriculture Organization of the United Nations. Indo-Pacific Fisheries Council. Proceedings—13th Session, Brisbane, Queensland, Australia, 14-25 October, 1968. Section II: Technical Papers. Pp. 206. (Bangkok, Thailand: IPFC Secretariat, FAO Regional Office for Asia and the Far East, 1970.) [67]

The Continuing Dialogue: A Bibliography for Convocation Honoring Dr Ernest Weber. By Professor James A. Goldman. (*Stacks*, No. 28, May 8, 1970.) Pp. 154. (Brooklyn, New York: Polytechnic Institute of Brooklyn and Society of Sigma XI, Polytechnic Chapter, 1970.) \$3. [77]

Annals of the South African Museum. Vol. 56, Part 2: A Review of the Geology and Palaeontology of the Plio-Pleistocene Deposits at Langebaanweg, Cape Province. By Q. B. Hendey. With an Appendix "The Langebaanweg Bovidae", by A. W. Gentry. Pp. 75-117+4 plates. (Cape Town: South African Museum, 1970.) R. 4. [87]

The Western Australian Museum. Annual Report 1968/1969. Pp. 63. (Perth: Western Australian Museum, 1970.) [97]

US Department of Health, Education and Welfare. Public Health Service Physicians-in-Residence: a Demonstration Project in Continuing Education. Pp. 9. Graduate, Then What—Jobs in Health: a Report on the Use of Radio as a Recruitment Tool for Health Occupations. Pp. 10. (Bethesda, Md.: Bureau of Health Professions, Education and Manpower Training, Division of Physician Manpower, 1970.) [97]

Mankind 2000. Pp. 22. (Binghamton, New York: James Wellesley-Wesley, c/o Center for Integrative Studies, State University of New York, 1970.) [97]

Smithsonian Contributions to Zoology, No. 46: Behavioural and Life-History Notes on Three Floridian Solitary Wasps (Hymenoptera: Sphecidae). By Karl V. Krombein. Pp. 26. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) \$0.40. [107]

Bulletin of the Florida State Museum—Biological Sciences. Vol. 15, No. 1: Courtship and Combat Behavior in *Gopheris berlandieri*. By W. G. Weaver, Jr. Pp. 1-43. \$0.75. Vol. 15, No. 2: Diagnostic Keys and Notes on the Damselflies (Zygoptera) of Florida. By Clifford Johnson and Minter J. Westfall, Jr. Pp. 45-89. \$0.80. Vol. 15, No. 3: Atlantic and Gulf of Mexico Menhaden, Genus *Brevoortia* (Pisces: Clupeidae). By Michael D. Dahlberg. Pp. 91-162. \$1.30. (Gainesville, Florida: Florida State Museum, 1970.) [107]

University of Tennessee—Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory. International Conference on The Photosynthetic Unit, May 18-21, 1970. Pp. xii+71. (Knoxville: University of Tennessee, 1970.) [107]

Resistance to Knowledge. By Hans Gaffron. Pp. 61. (San Diego, California: Hans Gaffron, 1970.) [137]

Smithsonian Contributions to Zoology, No. 47: New Ectocytherid Ostracods with a Key to the Genera of the Subfamily Ectocytherinae. By Horton H. Hobbs, Jr., and H. H. Hobbs, III. Pp. 19. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) \$0.35. [137]

Acta Oecologica Scandinavica, Supplementum 12: Impact of Man on the Scandinavian Landscape During the Late Post-Glacial. (Symposium on Oland, 11-12 June 1968.) Edited by B. E. Berglund. Pp. 103. (Copenhagen: Munksgaard, 1970.) [137]

Review of the Mineral Industry in Tanzania for the year 1969. Pp. 10. (Dodoma, Tanzania: Commissioner for Mineral Resources, 1970.) [137]

Indian Journal of Pure and Applied Mathematics, Vol. 1, No. 1, January 1970. Pp. 1-105. Annual Subscription inclusive of Postage: Rs. 36 (inland); 100s or \$12 (foreign). Single issue: Rs. 12 (inland); 33s 5d or \$4 (foreign). (New Delhi: National Institute of Sciences of India, 1970.) [167]

International Labour Office. Occupational Safety and Health Series, No. 22: International Classification of Radiographs of Pneumoconioses (Revised, 1968). Pp. 23. (Geneva: International Labour Office, 1970.) [177]

US Department of Commerce. National Bureau of Standards. NBS Special Publication No. 322: Photocopying Data Index, January 1965 through January 1970. Prepared by Photocopying Data Group. Pp. iv+84. (Washington, DC: Government Printing Office, 1970.) \$0.75. [177]

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Preparing for the Deluge

WITH all the heady excitement that went with the negotiation and signature of the Non-Proliferation Treaty, it might have been expected that the International Atomic Energy Agency at Vienna would have been transformed and even galvanized into unwonted activity. There is very little tangible sign of this in the annual report of the agency to the end of June and in the budget for 1971 and the accompanying programme which, sensibly enough, the agency has also published in anticipation of the meeting of the governors in September. To be sure, both documents are suffused with a sense of excitement of how different things will be in 1973, when the work load occasioned by the Non-Proliferation Treaty will lie squarely on the agency's shoulders. Outsiders may be forgiven for asking whether the agency is yet sufficiently aware of how different things will then have to be. Is there any hope that the monitoring of the Non-Proliferation Treaty can be carried out by a safeguards organization obtained by simple extrapolation from that which the agency uses at present for the inspection of the nuclear reactors voluntarily submitted to its care? Over the years, the scale of operations has grown steadily. In 1971, the agency expects to have 184 nuclear installations on its book compared with 156 in the current year and 130 in 1969. To be sure, research reactors and other such facilities still make up the bulk of the work. Next year, there will be 30 nuclear power stations to inspect, an increase of four. In the year to the end of June, the agency carried out 91 actual inspections of working installations, a mere 45 per cent of those which might have been carried out. Experience has shown that one inspection occupies an inspector for five days and that, in the year just past, an average of 1.6 inspectors is needed for each inspection, but the agency estimates that in the future its men will be kept more fully occupied as the machinery with which they have to deal becomes more complicated. The forward budget includes some wistful figuring about the likely productivity of the inspectors—the target seems to be about 100 MW per man year. The trouble, which must now be painfully apparent to everybody concerned, is that anything like a full blown inspection of the Non-Proliferation Treaty would require the mobilization of a small army of inspectors. The new budget does sensibly allocate some of the time of the inspectorate to training, but the accumulation of expertise likely to result will not match the need which full safeguards for the treaty will dictate.

This, no doubt, is why the agency seems to have put a great deal of faith in the work of the Committee

on Safeguards set up in June this year to hammer out the principles on which the inspection necessary for the Non-Proliferation Treaty should be carried out. By all accounts, the committee has singled out as one of its objectives the devising of a set of principles that will give the international agency a particular incentive to look closely at the nuclear operations of countries about to cross the threshold at which they might become nuclear powers in the military sense without letting them feel unfairly discriminated against. That is sensible enough as an objective, although it is important to acknowledge that a set of safeguards devised on lines like these is bound to vest in the international agency a degree of political responsibility that will lie uneasily with its present substantially judicial function. In short, it will have to be possible for the agency to turn particular attention, as circumstances change, from one group of countries to another. Is this the kind of role that international civil servants can assume? Or will there have to be some political forum in which decisions can be made about the points at which the safeguards agency should direct attention? Either way, the outlook is not particularly cheerful. The safeguards committee will need all the ingenuity it can muster to surmount merely this obstacle. Goodness knows what will happen when it comes to a decision about the cost of the safeguards operation and the partition of this among the signatories to the treaty. After all, it would seem to be grossly unfair that the nuclear powers immune from inspection under the terms of the treaty should be charged nothing for the cost of operating the safeguards procedure. On the other hand, small countries with no nuclear pretensions and not even a research reactor to call their own rightly feel hard done by to be asked to help to foot the bill. Before the committee's work is done, it will find itself counting the angels on the head of a pin.

In circumstances like these, it is not unacceptably smug to recall that the Non-Proliferation Treaty as a whole is more like an expression of good intention than an assurance that evil things will go away. Now that more than a score of countries have ratified it, and even nations like India are prepared to join in the argument about how it should be implemented for fear that too much insistence on the sovereign right to be a nuclear power will deprive them of a voice, there may be something in the view that even if the Non-Proliferation Treaty is not a guarantee, it is at least an attitude of mind. From that point of view, nobody should worry much if the IAEA's Committee on Safeguards is still in session in 1972—the important

thing will be that they are still talking. But there is of course a danger that too literal an interpretation of the expectation of those who sign the treaty may lead them, by then, to attempt more than they can possibly

accomplish. Not merely will that be bad for the Non-Proliferation Treaty but it will be disastrous for the IAEA. Falling flat on the face is not good for the public image.

Close Season for Technology

THE belief that Mr John Davies, the new Minister of Technology, would be in like a lion and out, quickly, like a wounded rabbit seems well on the way to frustration. So far, very little has been heard from him. To be sure, the holidays have now seized the government, and even the Secretary for Employment and Productivity, Mr Robert Carr, having uttered a gloomy warning about the prospects for the economy, has himself gone on vacation. But even if the Bank of England can be relied upon to manage the financial affairs of Britain in high vacation, it is beyond a joke that nobody should have acknowledged that a decade has passed since the need was first recognized for a radical reorganization of the government research establishments. And it is not merely sad but also destructive of people's confidence that the previous government's attempt either to solve or to conceal the problems of the research establishments, in Mr Wedgwood Benn's green paper, should have been allowed to lapse without replacement. Mr Davies, Mr Carr and even the Prime Minister might occasionally remember, while relaxing on their vacations, that a great many of those who work in government establishments are spending less comfortable vacations. Some of them may even be wondering whether they will have jobs to which to return.

So far, the new government (in the person of Mr Geoffrey Rippon, the five weeks' minister) is committed to producing a bill to set up a company for manufacturing nuclear fuel, the bread and butter item in Mr Wedgwood Benn's proposals for the Atomic Energy Authority. It may be too soon to be sure that this proposal will survive under the new government, but it has every promise of doing so. By common consent, there is no reason why the government should continue to finance with public money operations which can be controlled easily enough by routine legislation, and financed, in part at least, by private capital. If Mr Wedgwood Benn was in his time in favour of an element of denationalization in this field, who will expect Mr Davies to hang back? The real difficulties are those not merely of the remainder of the Atomic Energy Authority, with its development organizations and research establishments, but of the other government research establishments concerned with aeronautics, electronics and a miscellany of vaguely scientific activities accounting for the best part of a third of the whole of British expenditure on research and development. What is to be done about them?

Mr Davies should perhaps acknowledge that it is mistaken to contemplate the entire disbandment of the Atomic Energy Authority, and, since the mistake is

the responsibility of Mr Wedgwood Benn, it should be comparatively easy for him to do so. It is not sensible for the British government to relinquish a direct executive control on the conduct of research programmes in fast reactor technology, and there is no assurance that the arrangements made with the private consortia will be an adequate substitute. To be sure, if all goes well in fast reactor technology, it may turn out that the two estimable groups of companies now in the business will be able to construct the reactors which the Central Electricity Generating Board needs and will also be well placed to export copies of them. But if the course of developments goes badly, everybody will be in trouble and the government will find itself wishing that there were some means of stoking the fires. In other words, there is a chance that it will regret any outright abandonment of an autonomous research programme and that, if it does now entirely wash its hands of research and development in this important field, it may have to start all over again in the late seventies. What this implies is that the plans for the reorganization of the Atomic Energy Authority which may be hatched in the weeks ahead should leave some room for a substantial programme of research and development. £10 million a year, or a quarter of the net cost of the Atomic Energy Authority in round numbers, should just about meet the bill. Elsewhere among the government establishments, it will be widely acknowledged that Mr Davies has a serious problem. Few ministers would be able to sustain in the Cabinet a fresh claim on a share of the national resources as substantial as that now given over to the research establishments. Cost benefit analysis would be brought to bear, and it would turn out to be hard to justify the cost. But the government is also committed, morally and in some cases contractually, to the people employed in the government establishments. Moreover, there are brave experiments such as those at Harwell where it begins to seem as if a government establishment may yet be a spring-board for independent enterprise. (The biggest snag is that if Dr Walter Marshall is as successful as his backers hope, he is bound to be snatched away to other, less productive jobs.) The problem, in short, is how simultaneously to diminish the government's direct involvement in research and development, to increase the incentive which government expenditure can provide for industry to improve its techniques and at the same time to remain not merely just but generous to those who have individually signed on for a career in science at a government establishment.

Mr Davies should recognize that no solution is

possible without a better market for scientists and technologists. If these were normal times, he might calculate that the natural erosion of the labour force at Harwell and places like that would ensure that the last part of his problem went away. As things are, while industry is less able than it should be to employ skilled people, some method will have to be found for bridging the gap. What is to be done? Is there a case for seconding people without charge to industrial companies? Most probably the most serious argument against that is the antipathy which has grown up in the past few years between the young men from the business schools (or those who wish they had been to business schools) and the old men of the sixties and even the fifties who believe that good engineering is an end in itself. Yet in numerical terms, the supply of skilled people is all too small, and it also turns out that those who have not been to business schools have nevertheless earned their money by programming computers or dealing less articulately

than the younger people with the concept of what the business schools call a system. In short, one of the government's chief duties should be to recreate the sense that the vast reservoir of skilled people in the government establishments can help to solve a lot of problems, some of them economic and some of them social. It is true that talk of white-hot technological revolutions is unfashionable (but Mr Wilson might still have been in office if he had listened more carefully to what he said), but there must surely be some programme rather than a form of words to which even Mr Davies could set his hand. In practice, he would have to be prepared to spend more money on enabling industry to employ scientists than is at present spent on the same function in the establishments. So urgent is the need that the precise way in which the money is spent is less important than that it should quickly become available as a carrot that will tempt the civil servants away from the establishments that have outgrown their functions.

Whom Does the Science Adviser Advise?

THE discussion last week about the role of the President's Science Adviser in modern American government seems to have been timely, perceptive and polite. The first thing to be said is that the office is highly individual. Like other high officials of the Administration in Washington, the Science Adviser is not so much a policy maker as a courtier. Such influence as he may have will often be seen to rest on his personal influence with the President, and the more influential he is, the more fondly he is regarded by the scientific community. If all goes well—but this is exceptional—the Science Adviser even becomes at once the idol of his President and of professional scientists, and the time then comes, no doubt, when he begins to ask himself whether he should not also run for the highest office. But when things go badly, the boot is on the other foot. The President has no time for the advice he has to tender and the scientific community regards him as the cause and not merely the instrument of its own sense of isolation. In other words, being Science Adviser is a job whose rewards would be appealing only to very few people and whose discontents would drive saints to despair.

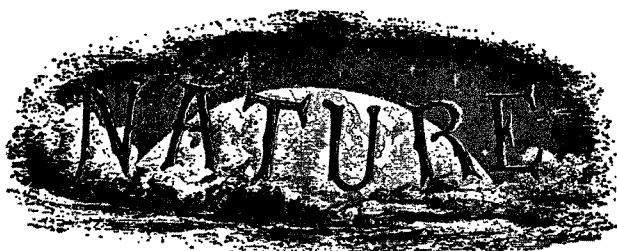
The fact that Dr Lee DuBridge has come in for round criticism of his handling of this office is not explicitly his fault. This seems to have been what Dr James Killian and his colleagues were saying last week. At the same time, it is only right to acknowledge that in a highly personalized job like this, the personal attributes of the Science Adviser—though not his virtues—should be called up for examination. Film stars are, after all, sometimes sacked from coveted parts because their flair is for playing some other part. And company presidents are constantly being told by shareholders that they have lost their touch. It is

therefore not to be regarded as in any sense remarkable that a scholar of the highest distinction and a scientist of the most widely acknowledged reputation should not at the same time be one of those adroit but bullying politicians whose survival is likely to be most certain in the present climate in Washington.

If Dr DuBridge has been less powerful than he might have been for lack of the qualities that have earned courtiers a bad name, it is also only fair to say that times have changed, so as to have transformed the role of the Office of Science and Technology. In the statements of Drs Killian and Wiesner last week, there seems to have been more than a thin streak of sentiment. Both of them, after all, were looking back on periods when a courtier's life was relatively successful—and even so they may have frequently glossed over some of their disappointments. As things have become in Washington, what the Administration needs is not so much a Science Adviser in the sense that Killian first devised but, rather, a hard-headed but experienced man who can knock together the heads of the heads of the agencies whenever necessary. This is the task which the Science Adviser at present carries out in his role as chairman of the Federal Council on Science and Technology. But Washington also needs a device for making sure that the opinions of scientists can be canalized effectively towards some central point. In the past, the Science Adviser, by being chairman of the President's Science Advisory Council, has been able to do this job effectively. When the President has both a need and a liking for what his courtiers have to say, PSAC is a venerable institution. Now, with all the tensions that abound and with the growth of the profession of science, it would be much easier if the focus of the

scientific community's interest in the government could be elsewhere, chiefly at the National Science Foundation with informal links with the Pentagon and the other agencies. So long as there persists this conflict between the role of the Science Adviser as courtier and as lightning conductor, criticism of him and his office will persist. This is the point on which the congressional enquiries should concentrate.

100 Years Ago



Dictionary of Scientific Terms. By P. Austin Nuttall, LL.D., editor of "The Classical and Archaeological Dictionary," "Standard Pronouncing Dictionary," and numerous Educational Works. (London: Strahan and Co., 1869.)

WE are thankful to be able to say that we know nothing more of Dr. Nuttall than we learn from his title-page. We never even heard of him till we read his book, and we most sincerely hope that, as an author of scientific works, we shall know him no more. What the "numerous educational works" that he has published are we cannot tell, but if they are at all like his "Dictionary of Scientific Terms," the sooner they are consigned to the trunk maker and the butter-man, the better will it be for the welfare of the unhappy youths for whose benefit they were composed. These may seem hard words to apply to a writer who hopes to "receive every indulgence from a generous public" (p. xi.); but when that writer outrages all our better feelings by stringing together a series of idiotic absurdities, and calling the result a "Dictionary of Scientific Terms," how are we honestly to deal with him, except by exposing a few of his blunders? We will begin by testing his chemical knowledge. It will hardly be believed that he regards black-lead, brass, magnet, ochre, pewter, and steel as constituting "a few of the principal metals" (p. xviii.); that nitrogen is "unrespirable" (p. 230), although he has previously told us at p. 12 that it forms about 80 parts of atmospheric air; that oxygen "generates acids" (p. 239); that alum is "an earthy chalk, a sulphate of alumina or of potash" (p. 18); and that "culinary, rock, or sea salt, is chloride of soda" (p. 277). His natural history is as peculiar as his chemistry. We will merely put his zoology to the test, assuring our readers that in so far as accuracy is concerned, his zoology, botany, and mineralogy are much on a par. "Zoology," he tells us, "embraces an account of all animal creation, the principal classes being the *Mammalia*, *Aves*, *Reptilia*, *Pisces*, *Invertebrata*, and *Insects*." The first class is subdivided into nine orders, of which one is "*Edentata*, or animals wanting some of the teeth of other animals" (p. xiii.) Being anxious to learn more of these covetous, commandment-breaking creatures, we turned to *Edentates*, and found that they are "an extensive order of the class *Mammalia*, comprehending those unguiculated quadrupeds which have no front teeth, and divided into three tribes, the *Tardigrada*, the ordinary *Edentata*, and the *Monotremata*." We leave our zoological readers to decide how far this description is an accurate definition of the order, according to recent views, such as our author might have learned by consulting the works of Owen, or of Huxley.

From *Nature*, 2, 333, August 22, 1870.

OLD WORLD

ROCKETRY

Britain's Space Gold

A GOLD-PLATED sphere 76 cm in diameter will be the first satellite to be launched by a British rocket, as distinct from satellites launched for British scientists by the United States. With the usual proviso that all goes well, the satellite will be orbited on September 1 from Woomera. Together with the 13 kg satellite, equipment weighing 69 kg to check the performance of the rocket and the spent third stage of the rocket itself (42 kg) will go into orbit.

The three-stage Black Arrow rocket is a direct descendant of the Black Knight test rocket and it had its first firing in June last year. That was only a partial success, but it was repeated in March this year, when the rocket passed its test with flying colours. Next month's firing will therefore be the first orbital attempt—it was postponed from 1969 because of the initial failure.

Roughly equivalent to the American Scout rocket in that it will be able to handle payloads up to about 150 kg, the first and second stages of the Black Arrow burn hydrogen peroxide and kerosene, and the third stage has a solid propellant.

Although the first satellite, to be known as Orba, is diminutive as satellites go nowadays (China's first satellite weighed 380 kg, although Japan has orbited a 25 kg satellite) it apparently fills something of a gap in the scientific hardware in space that has already been noticed by COSPAR. The fine finish of Orba and its elliptical polar orbit make it ideal as a probe for measuring air densities and for observations of the motion of the upper atmosphere. Mr Desmond King-Hele and his colleagues at the Royal Aircraft Establishment, Farnborough, were the first to discover from observations of satellite orbits that the upper atmosphere must on average be rotating faster than the Earth. In fact the speed of the atmosphere varies from 1.4 times that of the Earth at heights of 400 km down to 1.1 times the Earth's speed at 200 km, and seems to have been detected at lower heights still (see for example D. G. King-Hele, *Nature*, 226, 439; 1970). Thus there is a dominant west to east wind at high altitudes that will be acting nearly at right angles to the plane of the orbit of Orba. (The orbit will be inclined at 82° to the equator.) The perigee and apogee heights, 350 km and 1,100 km respectively, make the orbit a compromise between a low orbit that would be strongly influenced by the rotation of the atmosphere but would lead to rapid re-entry, and a higher orbit for which the effect of the atmosphere will be smaller. The Farnborough group will be using optical and radio measurements of the change in inclination of the orbit to add to what is known about the rotation.

Between now and 1973, two or possibly three more launchings in the Black Arrow series of satellites are expected. The second satellite in the series planned for next year will be chiefly a technological satellite to test such things as an altitude control system, solar cells and new surface finishes, but it will also include a micrometeoroid experiment from the University of Birmingham. The third satellite, in 1973, will carry meteorological sensors, and it is said that there may be an additional satellite fitted in during 1972.

OPEN UNIVERSITY

Probation plus Costs

by our Education Correspondent

LAST week's announcement that the government is prepared to let the Open University enrol 25,000 students for next year does not signal an end to the university's fears for its future. But it does mean that the Open University will have a good chance to prove its worth before a final decision is reached by the Department of Education and Science. What the announcement made clear is that the university will be given a grant-in-aid to go ahead with its present plans, but that the government will discuss with the university authorities "the future scale of its operations, the contribution that it can make to the development of higher education provision in the future, and the prospects of increasing its revenue with a view to limiting the level of financial support from public funds". In other words, the university is on probation, and even if it comes up to expectations, it may have to take more of the financial burden on its own shoulders.

The cost to the Exchequer of running the Open University has not yet been worked out, but an early estimate suggested that the minimum basic costs—buildings, administration and so on—would be about £4 million each year. It now seems that the government has realized that the savings it is looking for will not be made simply by limiting the university's intake—for one thing, the chief costs are broadcasting time, tutoring and central administration, all of which are relatively insensitive to changes in student numbers—and it has therefore decided to see whether the university can at least pay some of its own way.

The university itself is not unduly alarmed by such suggestions, and it has already started to explore ways to take advantage of its unique position. The most promising source of finance is its own software, and the university authorities claim to have received expressions of "very high interest" from foreign universities and governments for the television and audio tape recordings of its foundation lecture broadcasts. There is also the possibility of selling books of correspondence teaching material—more than 100 have already been produced—but perhaps the most promising money spinner is the so-called MacArthur microscope which has been developed for the foundation course in science. The microscope packs into a small case, is powerful enough to cope with all the requirements of an undergraduate course, and will probably sell for about £10–£15. More than 8,000 microscopes will be required next year by Open University students alone, and the university's solicitors are at present trying to get patent rights on the instrument.

Other possible sources of finance are endowments and course fees, but the university is anxious not to raise its charges, because it does not want to discourage potential students. Even with the fees set at £140 for a BA degree and £180 for a BA with honours, the university succeeded in attracting chiefly middle-class students, while applications from manual workers and technicians were few and far between. The university will now be hoping for a wider cross-section of applicants for 1972 and for a drop-out rate of less

than 50 per cent, so that it can convince the government that it has an important contribution to make to the provision of higher education in Britain.

RABIES

Reprieve for Pets in Sight

IN recommending that dogs and cats should once again be allowed into Britain, provided that they are held in quarantine for six months and given at least two shots of anti-rabies vaccine, the committee of inquiry on rabies has settled for something slightly less than absolute safety. Whatever the merits of the complete ban on imports imposed last March, even that measure could not rule out smuggling by people loath to part with their pets, and in its interim report (HMSO, London, 1s 9d) the committee has decided that the small risks of controlled importing are preferable to the dangers of an untraceable source of the disease should a rabid animal enter the country illegally. But the ban will continue for other feline and canine species until the committee has made up its mind about the kinds of precaution that would be needed.

British quarantine regulations have already changed twice in less than a year. Last December, the length of quarantine was increased from six to eight months, following the case of a rabid dog which bit several people in Camberley soon after coming out of kennels, and the final ban was prompted by a dog which died of rabies at Newmarket nine months after arriving in Britain. At that time it was argued that six or eight months' quarantine was not safe enough, that longer periods were impractical because there was insufficient room in kennels, and that vaccination was too unreliable to be used instead. The combination of measures that the committee would like to see is strong enough to overcome all these arguments. Only inactivated vaccines would be used, and each batch would be rigorously tested for potency; animals would be vaccinated as soon as they arrived at their kennels, and again one month later; and records would be kept for nine months after release of where each animal was living. The committee has made a few preliminary suggestions for improving the isolation of animals inside kennels, but it will explore this question more fully—together with the precautions that should be taken if an outbreak of rabies occurred in the wild—in its final report.

Several myths about rabies seem to have been demolished by the scientific evidence that the committee has heard. There are no signs of the "important new strain" of rabies virus that the popular press was fond of earlier in the year, and neither is there any indication that the incubation period is tending to grow longer. On the other hand, the possibility that the virus might be transmitted indirectly, without a bite, from one animal to another within a kennel is still not ruled out. It is now, however, up to the Minister of Agriculture, Mr James Prior, to decide whether he thinks that the recommendations of the committee are strong enough to keep the enzootic rabies of Europe on the other side of the English Channel; and it will presumably be up to future Ministers of Transport to satisfy themselves that any tunnel under the Channel would be impenetrable to foxes.

CHOLERA EPIDEMIC

Tip of the Iceberg shows

THE cholera epidemic in the south of the Soviet Union, which seems seriously to have interfered with holiday traffic around the Black Sea, and which is being treated with great solemnity by the public health authorities in the Soviet Union, is by no means unexpected. Since the early sixties, it has been plain that endemic cholera has been spreading through India, Pakistan, South-East Asia and the Soviet Union. Although the number of known cases of cholera fell from more than 100,000 a year in the late fifties to less than 50,000 in 1960, the outward spread of the El Tor organism from Celebes in 1960 seems to have marked the beginning of what the World Health Organization has called the seventh epidemic of cholera (the sixth supposedly having lasted for the first quarter of this century). The countries first affected were those of South-East Asia and the region around the Himalayas, north and south, but even Japan has been affected on two occasions in the past decade.

Progress in the treatment and prevention of cholera, while steady, remains slow. With good diagnosis and facilities, the death rate of infected persons can be reduced from 60 per cent to 1 or 2 per cent, while there has been heartening if predictable progress in the treatment of various complications such as those of the liver. One of the difficulties in the prevention of the disease has been the comparatively short duration of the immunity conferred by the vaccines developed in the past few years. For one thing, vaccination seems to be effective only in roughly half of those treated and its effects persist only for six months or thereabouts. An expensive series of controlled field trials sponsored by the World Health Organization in the past six years has so far yielded no spectacular improvement of vaccine prophylaxis. Much work remains to be done on the identification of cholera carriers in the population, while the epidemiology of the disease and the incidence of immunity in populations in which cholera is endemic requires attention only now being devoted to it.

ATOMIC ENERGY

Rising Budget for IAEA

THE doldrums appear to be over for the International Atomic Energy Agency in Vienna, which is now counting on having \$13.78 million to spend in 1971, an increase of 10.1 per cent on the year. The agency's work for the safeguarding of nuclear power installations, and its planning for the time (two years ahead) when the Non-Proliferation Treaty will have to be policed, account for much of the increase. In 1971, safeguards procedures will indeed take 11 per cent of the total budget, at least if this is approved when submitted to the board of governors of the agency in September.

Elsewhere in the pattern of the agency's spending, the outlook is not as cheerful. The technical assistance programme, on which a great many underdeveloped countries have relied and to which many others look for technical help with the development of nuclear industries, will have an extra \$16,000 or just over 2 per cent more to spend next year. Much the same is true

of the other items in the proposed budget—the Centre for Theoretical Physics at Trieste, for example, will have to look for any increases of expenditure to other organizations than the agency.

One striking feature of the budget document now circulating is the forecast which it includes of expenditure in the period 1971–76.

ANTARCTIC

Russia moves West

by Our Soviet Correspondent

THE latest projected Soviet research station in the Antarctic—the Leningradskaya—represents a new venture in the Russian exploration of the continent. For the first time, a Soviet expedition will be operating in the western area, on Ots Bay, outside their traditional eastern sector. This change, according to the deputy head of the Soviet Antarctic Expedition, V. M. Rogachev, is part of a programme to obtain more complete data about the whole Antarctic continent, and it will be followed by the establishment of a further, as yet unnamed, “bridgehead” on Cape Dart. Russia will then have a series of seven research stations, spaced at approximately equal intervals around the periphery of the continent.

An advance party of six, headed by the distinguished polar scholar, A. B. Budretskii, will travel out to the Leningradskaya site, as part of the sixteenth Soviet Antarctic Expedition, preparations for which are now well under way. This expedition, which will comprise in all some 300 persons, is to undertake a comprehensive programme of research, including a continuation of the series of deep bores into the ice-cap, already successfully initiated by previous expeditions.

PHYSICS

Following in Weber's Footsteps

Two groups in England are among those preparing to test Professor Joseph Weber's claim to have detected gravitational radiation. Neither Mr P. Aplin at the University of Bristol nor Professor W. D. Allen's group at the Department of Applied Physical Sciences at the University of Reading seems dismayed that Professor Weber's work has not been without its critics, and that the astronomers are hard pressed to account for the huge flux of gravitational radiation that his experiments, taken at face value, have detected. Both take the view that Professor Weber's work needs to be independently tested.

Professor Allen and Mr Aplin both began thinking seriously about setting up detectors for gravitational radiation in June last year. Mr Aplin expects to have his working within a few months, but Professor Allen's may take a little longer. Both pieces of equipment are roughly along the lines of that used by Professor Weber at the University of Maryland, and at the Argonne National Laboratory, and they will both be tuned to the same frequency, 1,661 Hz. In the first instance Mr Aplin and Professor Allen will be looking for coincidences between the signals picked up by their detectors—later they hope to cooperate with Professor Weber and other groups to establish a world-wide net to catch gravitational waves. Both have financial support from the Science Research Council and from their own universities.

Chips off the Plates

THE twelfth leg of the zigzag voyage of the deep-sea drilling vessel *Glomar Challenger* around the oceans of the world "achieved more than I ever expected", Dr Anthony S. Laughton of the National Institute of Oceanography, Surrey, said in London last week. Dr Laughton, who was co-chief scientist with Dr William A. Berggren of Woods Hole Oceanographic Institution, Massachusetts, for the fifty-five day cruise which ended in Lisbon on August 11, was presenting the first scientific reaction to the three thousand feet of cores that were collected. Leaving Boston on June 17, the ship described an arc up to 60° north that took in thirteen drilling sites at the edge of the continental shelf off Newfoundland in the Labrador Sea, on the Reykjanes Ridge south of Iceland, the Rockall Plateau and in the Bay of Biscay.

This latest cruise of the *Glomar Challenger* is the first with a co-chief scientist from outside the United States, reflecting the growing internationalization of the programme. The trend continues on the next leg, which is covering the Mediterranean and which ends at Lisbon on October 6. Cruises have been mapped out until August 1972 when the additional support for the programme that the National Science Foundation awarded last year comes to an end. Following the exploration of the Mediterranean, the *Glomar Challenger* will take a more southerly route across the Atlantic to Puerto Rico for investigations in the Caribbean, and will then follow a devious circumnavigation taking in areas of geophysical interest in the northern Pacific, the Indian Ocean, and the south Atlantic.



The drilling vessel *Glomar Challenger*.

Although Dr Laughton was speaking only days after the conclusion of leg twelve, he was able to report several notable achievements of the cruise. From the technical point of view it had been pleasing that deep-sea drilling had proved feasible up to 60° N in the north Atlantic. Only half a day's drilling out of fifty-two had been lost because of the weather. Using

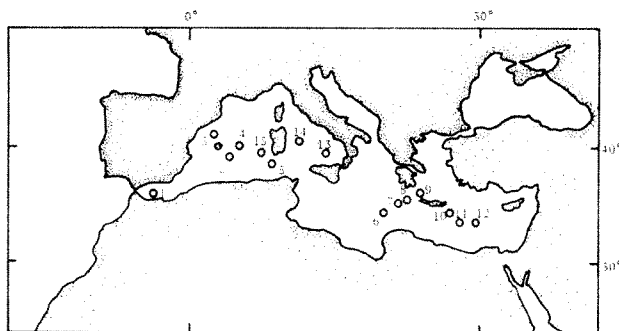
techniques developed for the ill-fated Project Mohole, the *Glomar Challenger* stays within a circle of a 100-foot diameter for the several days that it takes to complete a drilling.

Scientifically, the cruise has collected cores from several fragments of continental material left behind by the opening of the north Atlantic, has shown that the Gulf Stream once flowed into the Labrador Sea, and unexpectedly has revised the date of the commencement of the last glaciation. The continental fragments are evidence that the break between North America and Europe was not as clean as that producing the jigsaw fit between Africa and South America. Greenland was also left behind, but Iceland is a purely volcanic island built up from the mid-Atlantic ridge. To begin with, cores were recovered from Orphan Knoll, an isolated seamount just beyond the continental shelf near Newfoundland, and the deepest core recovered was a black sandstone containing fragments of anthracite that might have been laid down in a river bed, showing that the seamount was once a low-lying continental fragment. The same kind of evidence was unearthed from the Rockall Plateau, and Drs Laughton and Berggren say that they have enough data from two boreholes to give a chronological history of the sinking of the plateau after it separated from Greenland 56 million years ago. But why these two regions should be disappearing into the seabed—at a rate of one foot every thousand years for the Rockall Plateau during the last of the three stages of sinking that have been identified—is still a mystery.

Two boreholes in the Labrador Sea contained layers of rock fragments, at a depth corresponding to 3 million years ago, that are believed to have rained down on the seabed from melting icebergs. Dr Laughton said that this is an earlier date than previously thought for the beginning of the last glaciation in the North Atlantic. It corresponds to the middle of the Pliocene, and is the first strong evidence that the glaciation was earlier than the boundary between the Pliocene and the Pleistocene, 1.8 million years ago. Beneath the glacial debris, the cores contain subtropical organisms showing that before the glaciation the Gulf Stream was probably flowing along the Labrador coast.

The drillings on the flanks of the Reykjanes Ridge have demonstrated that the thick sediments there are due to turbidity currents depositing large amounts of material and do not have to be interpreted by variations in the rate of seafloor spreading. In the Bay of Biscay the intention was to find how long ago the bay opened due to the rotation of Iberia away from France, but so far the data from two boreholes are inconclusive.

In the Mediterranean, the scientific crew of *Glomar Challenger* will be collecting cores from a closing sea to compare with those from the Atlantic. The co-chief scientists, Dr Kenneth J. Hsu of the Swiss Federal



Proposed boreholes in the Mediterranean.

Institute of Technology in Zurich and Dr William B. F. Ryan of the Lamont-Doherty Geological Observatory, will be investigating the formation of Sardinia, Corsica and the Italian peninsula, and looking for evidence that the eastern Mediterranean was once a large sea that has been crushed as a result of the spreading of the Atlantic and Indian Oceans.

But, at present, drilling is hampered by what the members of the crew call the "chert barrier"—layers of chert, a rock similar to flint, that have turned out to be unexpectedly abundant beneath the seabed. The chert blunts the drill bit surprisingly quickly, and when that happens drilling in that particular borehole has to finish. Once the dulled bit has been retracted there is no way in which the entrance to the borehole can be found again. This is why the permanent members of the deep-sea drilling project are still rejoicing over a test carried out off New York just before leg twelve in which it was proved that when the *Glomar Challenger* is equipped with a high resolution sonar the drill can be guided into an old borehole if the borehole is capped with a metal cone 16 feet in diameter to direct the drill. This technique will be used in earnest for the first time on leg fifteen in the Caribbean when the ability to replace worn bits will allow deeper drilling than before. During leg twelve the deepest core came from 3,000 feet below the sea floor, and drilling was conducted in water depths up to 16,000 feet.

Can the IAU hold its Head above Water?

from our Special Correspondent

Brighton, August 18

It will be interesting to see if the fourteenth General Assembly of the International Astronomical Union, now in session here, can avoid being swamped by its participants. 1,800 astronomers, representing all branches of their discipline, and their 500 guests have descended on Brighton for the assembly, which takes place every three years. The choice of this part of southern England is recognition of how astronomy has blossomed here, at the nine year old University of Sussex and at the Royal Observatory close by near Eastbourne. On Monday evening during the registration formalities which everybody had to go through, there were signs of chaos, in spite of the computer printouts of participants' names that were being used. Perhaps that was to be expected at the start of a conference of this size—people were saying that Prague was worse three years ago.

Nevertheless, nearly everybody in astronomy is here, and on this basis the assembly cannot fail to be remembered as a success. All the same, old astronomers may lament the day when a general assembly of the IAU really was something. Then people might travel for days or even weeks on a boat for a rare chance to talk with other people in their subject. For a great many astronomers, international conferences are two a penny nowadays. That is why one of the resolutions to be introduced at the opening assembly was that, after 1973, general assemblies should be at four year intervals. It still holds true, however, that astronomers working at small remote observatories which tend to be cut off from the mainstream abroad probably value assemblies more.

That is one of the reasons why the discussions of the forty or so commissions into which the IAU has divided astronomy—the main business of the general

assembly—are so important. Before the assembly, the president of each commission writes a draft report of what has been happening in that particular subject during the past three years. These draft reports, which are polished into a final version by discussion at the commission's meetings, can be very good—the best are read by astronomers wanting to brush up their knowledge of a particular field. Much depends on the character of the president, however, and some allow their meetings to degenerate into a discussion of what is to be discussed. "Sitting there, filling their waistcoats", is how one astronomer described it, lamenting the preoccupation with organizational problems at some of the commissions.

Even if a general assembly is less interesting than the small congresses on specialist topics that the IAU is organizing all the time, the IAU as a body is of inestimable value to astronomy. It smoothes a way for the international exchange of astronomers that would otherwise be fraught with political difficulties. It is responsible for the telegraph service that informs astronomers around the world of important news—the discovery of supernovae, new pulsars, comets and the like. It concerns itself with the protection of radio astronomy bands from interference. Most important is the work the IAU does for international collaboration, vital for such studies as long baseline interferometry and source surveys.

A nice question with political overtones has blown up about where the 1973 assembly is to be held. The Russians suggest Warsaw to coincide with the 500th anniversary of the birth of Copernicus in Poland, but the Australians want Sydney. The problem is that by IAU rules the invitation has to come from the host country, and Poland so far has not indicated what it wants.

NEW WORLD

Grumbles at Dr DuBridge

THE Office of Science and Technology and the role of its chairman, the President's Science Adviser, have once again commanded the attention of the House Subcommittee on Science, Research and Development, which is conducting hearings on the matter of whether the United States should pursue a national science policy, whatever that may be. Two former science advisers who appeared before the committee last week, Dr James R. Killian and Dr Jerome Wiesner, defended by implication the present incumbent of the office, Dr Lee DuBridge, who has been accused of failing to protect the interests of the scientific community. Other witnesses, however, had harsh words for Dr DuBridge and his office.

Dr Edward U. Condon, for example, professor of physics at the University of Colorado, bluntly referred to the "dwindling influence" of the President's Science Adviser and to the fact that three months ago, when major changes in the White House staff were announced "the Science Adviser and his Office of Science and Technology were not shown at all in the organization chart as published in the *New York Times*". Failure even to mention the office indicated to Dr Condon that "the President is unaware of the importance of science in the world today. This strikes us all the more forcefully in the light of experience in the past 18 months, in which partisan political considerations seemed to dictate choice of individuals for major appointments in government science agencies". Dr Condon may have been thinking of the case of Dr F. Long, whose nomination as director of the National Science Foundation fell through when his attitude towards the antiballistic missile system became known to the President. The general theme, if not this specific instance, was certainly on Dr Condon's mind, for later in his testimony he warned that "many scientists feel that there are now signs again of renewed repressive tendencies like those which were so strong in the 1952-60 period".

Dr Condon's disappointment in the Office of Science and Technology was shared by Dr Raymond Bowers, professor of physics at Cornell University, who told the committee that in the past few years the office "has not played the strong role that is necessary if we are to introduce better management into the federal science enterprise". Dr James Killian, too, remarked that although the Office of Science and Technology has sought to moderate cutbacks, "the fact remains that an accumulation of uncoordinated actions has brought disorder and dismay in the house of science". But both Dr Killian and Dr Wiesner made clear that the loyalties of a president's Science Adviser are to the President and not the scientific community. Asked if he saw any need for a reorganization of the Office of Science and Technology, Dr Wiesner explained that because of the size and complexity of the Cabinet offices, "cabinet officers become more advocates of their agencies, no matter how hard they try not to, than impartial agents of the President, so the President

finds himself a captive of these organizations. The most important problems of the President's Science Adviser and the President's Science Advisory Committee are to act as assistants and see that the President has had a full range of advice". Dr Wiesner added that there were those who thought his job should have been to represent the scientific community to the United States Government; "I felt my role was to ensure the healthiest possible scientific activity for the country's technological efforts, but this is very different from being spokesman for the scientific community".

Dr Killian agreed that the mechanism for advising the President on scientific affairs "ought to be built to serve the President in a way that is not caught up in the interdepartmental advocacy . . . and to give objective advice that is unrelated to the partisan interests of given departments".

Several witnesses have dwelt on the plight of graduate education and their number was added to by Dr Wiesner, who wanted to see the responsibility for higher education separated from the lower kinds and embodied in a National Institute of Education, which might become part of the National Science Foundation. Dr Bowers hailed as the great need of the next decade "the construction of a more rational and comprehensive system of support for graduate education in its own right and the moving away from a situation where graduate education is largely subsidized as a beneficiary of research support". Dr Bowers, too, believed that the National Science Foundation was best suited to administer the support of graduate education in the sciences.

Other reorganization plans pressed on the committee included Dr Condon's proposal that Congress should set up a Joint Committee on Science Policy which would oversee the relationship between science and other fields such as the health services and the elimination of war. Another joint committee, to be called the Joint Committee on Science and Technology, was proposed by Mr Pat Haggerty, the chairman of Texas Instruments, who envisaged such a committee as being the Congressional counterpart to a stronger Office of Science and Technology. Mr Haggerty believed that the present office does a creditable job considering that it has only twenty professional staff, but in a better world the office would be folded into a much larger Council of Science and Technology Advisers, similar to the Council of Economic Advisers.

It was Mr Haggerty who made perhaps the cleverest speech (though not necessarily the most illuminating) that the committee has yet been treated to. Not for him the ritual wringing of hands at the golden age that is no more. He launched his audience straight into an economic analysis which revealed that the United States has a favourable trade balance in only one of four kinds of product, those that are "technology intensive". But in this crucial sector the country's trade with its two chief competitors, Western Europe

Why Science is in a Mess

"FEDERAL reductions and shifts in science support appear to have been taken without the integrative guidelines of a policy consensus. Each of these actions might have seemed reasonable within the narrow context in which it was decided, but they are now having a cumulative effect which threatens to be very damaging not only to American science but to our universities. Taken together, these actions may curtail the amount and quality of basic research. They may diminish our capacity to educate scientists and engineers. They threaten to discourage young people from electing science and engineering as fields of study. They threaten the breakup of experienced teams of talented scientists and the closing of facilities. They threaten to erode the pre-eminent position of the United States in science and technology. They provide a telling example of the need for new policies which can help to reduce uncoordinated federal decisions.

"These federal actions include Section 203 of the Military Procurement Authorization Act for 1970 [otherwise known as the Mansfield amendment which prevents the Department of Defense from supporting basic research not obviously relevant to its needs]; abrupt changes in federal student aid programmes, such as the cutback in the National Science Foundation's traineeships; the reduction of the National Defense Education Act graduate fellowships and those of the National Institutes of Health which may be phased out; the phasing out of the NASA traineeship programme and the sustaining university programme without replacing them by other programmes to prevent rocking the boat; the expenditure ceilings imposed on the National Science Foundation; NIH's negotiated reductions in grants; the possible curtailment of funds for the national defence student loan programme; and the Administration's proposal for a family income ceiling of \$10,000, above which students could no longer receive interest subsidies (despite high continuing interest rates) for guaranteed loans.

"It is exceedingly important to look at the additive effect of all of these actions, some initiated by Congress and some by the Executive Branch. The total impact of these independent, unrelated actions is not only affecting the high level of funding available, but it is introducing a high degree of instability and uncertainty in the conduct of basic research and in its fiscal planning; and there is in addition an adverse financial impact on the entire university. It is eroding the carefully nurtured partnership of the Federal Government and our universities which has been so spectacularly successful over the thirty years."—Statement by Dr James R. Killian to the House Subcommittee on Science, Research and Development on August 11, 1970.

and Japan, declined from a favourable balance of \$1,600 million in 1962 to only \$700 million in 1968. Based on present rates of growth in population and gross national product, Western Europe and Japan may attain a GNP per capita as large as or even greater than that of United States (\$8,500) by the year 2000.

"Need I say more?", Mr Haggerty could almost be heard to ask at this stage, but he went on to spell out his belief that further improvement in the national quality of life depended on improving the national competence in science and technology, whereas total research and development activity in the United States had in fact declined, from its highest ever level of \$25,400 million in 1967 to an estimated \$27,250 million in 1970. This may not look like a decline, but of course \$27,250 million received in 1970 is worth only \$23,250 million in the currency of 1968.

How much money should be devoted to science, academic and otherwise? Mr Haggerty thought that the recent decline in support was inevitable, granted the pressing nature of society's other needs, but the erosion of scientific research should not be allowed to proceed any further. An increase in the federal support for science of about 6 per cent a year would take account of inflation and even allow a little real growth to occur. Mr Haggerty thought that a reasonable increase of this nature should be guaranteed for the next three years or so, because of the long range nature of science and so as to avoid the kind of aberrations in support that underlie the present problems. Just what those aberrations are and the damage they have brought about, nobody has explained more succinctly than Dr Killian (see this page).

ABM

Boondoggle or Bargaining Chip?

from our Washington Correspondent

ONCE again the Senate has been bullied by the Administration into allowing the deployment of the Safeguard anti-ballistic missile system to proceed as planned. Last week, which marked the end of the second round of SALT talks at Vienna, the Senate rejected by a margin of five votes a proposal that would have restricted Safeguard to the two bases at which work has already begun. Unless a slightly milder version of the same proposal is passed this week, the Pentagon will be able to build Safeguards at two additional sites, making four in all.

Since last year's Senate debate, when Safeguard was saved by a single vote, the emphasis of the argument has shifted from whether or not the system will work to whether it will be a useful bargaining counter at the SALT talks. The government is sufficiently persuaded of the system's importance to have twisted the Senate's arm with a last minute telegram from Ambassador Gerald Smith, the United States negotiator at the SALT talks, implying that without the prospect of Safeguard being built the Russians might lose all interest in the arms talks. The telegram, whose contents have not been revealed, is said to have swung the debate against the proposed limitation of the Safeguard system. The views expressed in the telegram, whatever they may be, have aroused the

suspicions of Safeguard's opponents because in testimony before a Senate committee earlier this year, Ambassador Smith strongly implied that he had enough bargaining leverage without any extension of the Safeguard system.

What with the technical and diplomatic complexities of Safeguard, and with much of the relevant information unavailable, a natural inclination would be to give the government the benefit of the doubt and let it do what it thinks best. Any such confidence is tempered by the fact that the Pentagon planners seem to have changed their minds several times about what the system is supposed to do and how it is to do it. Yet while the task of Safeguard has changed, its hardware is still based on the Nike-Sentinel system which was rejected as inadequate when first proposed. It is little wonder that many independent scientists, including three of the four former science advisers to the President, have gone on record as opposing the system whether for its technical deficiencies or other reasons (see *Nature*, 226, 893; 1970). In last week's debate there emerged further signs of disenchantment with Safeguard among the scientific community, together with evidence that the Pentagon is beginning to share some of their scepticism, at least to the extent of devising a new version of the Safeguard system known as Project Hardsite.

The basic elements of the system, the fast Sprint and Spartan missiles, and the perimeter acquisition (PAR) and missile site (MSR) radars, were originally designed to defend cities against a light Chinese attack, although from quite early on there seems to have been the option of upgrading the defence to meet an attack on cities by the Russians. Early in 1969, however, the Sentinel system, as it was known, was rechristened Safeguard and sold to Congress as a means not only of defending people against the Chinese but also of defending Minuteman missiles against the Russians.

This position smacked of commonality, the concept by which Mr McNamara tried to thrust the disastrous F-111 aircraft down the throats of both the Navy and the Air Force, and last month the powerful Senate Armed Services Committee persuaded the Pentagon to drop the idea of building Safeguards to defend against a Chinese attack on cities and to concentrate instead on protecting missile sites. Meanwhile critics had begun to argue that the heart of the Safeguard system, the missile site radar (which together with its ancillary computers costs the best part of \$200 million), could easily be knocked out by Soviet SS-11 missiles (roughly the equivalent of Minuteman missiles), which would leave the 150 or so Minutemen under the MSR's protection defenceless against attacks by SS-9 missiles. In their present form SS-11s cannot knock out a Minuteman in its silo, but the giant SS-9 missiles, which the Russians began redeploying in June, are believed to carry three 5-megaton warheads whose re-entry pattern happens to coincide with the spacing of the Minuteman silos. Moreover, the Soviet Union has begun to equip its SS-11 missiles with multiple warheads (a test firing with two dummy warheads was reportedly made earlier this month), which would raise the number of Sprint interceptors required to defend the MSR.

These Soviet moves have been accompanied (some say caused) by the United States' decision to start deploying multiple warheads on its Minutemen as

from June and in its Poseidon submarine missiles from December this year. The effect on the ABM debate has been for the supporters of Safeguard to point out how badly it is needed and for the critics to claim that it would not work against this kind of opposition; moreover, since the Russians know this, Safeguard is useless even as a "bargaining chip" which might be dropped in return for the Russians cancelling their SS-9 programme. The point was most succinctly put in the Senate debate by Mr Young of Ohio who said, "The Nixon Administration has claimed the Safeguard is absolutely essential as a defence against the Chinese. How then can they justify trying to trade it away to the Russians? If it is an essential system, we should keep it; if, as appears to be the case, it is a worthless boondoggle, the Russians surely know it and there is nothing to be gained by deploying it for bargaining purposes. Safeguard is not the billion-dollar poker chip the administration claims it is."

Much of the disquiet about the system's capabilities has sprung from the O'Neill report, a study of Safeguard by independent scientists that was convened by Dr John Foster, the Pentagon's Director of Defense Research and Engineering. Members of the study group included Dr Marvin L. Goldberger, until recently chairman of the President's Science Advisory Panel on Strategic Weapons, Dr Sidney Drell, also a member of the panel, and Dr Lewis M. Branscomb, director of the National Bureau of Standards.

The burden of the group's report, which was delivered to Dr Foster and Secretary of Defense Melvin Laird in January but not made public until last week's debate, was that if the only purpose of Safeguard was to defend the Minuteman silos, the project should proceed no further. In its place a "dedicated" system should be developed which would have smaller and cheaper radars than the MSR, with each radar defending fewer silos. The Department of Defense had clearly reached a similar conclusion, because included in the \$1,027 million being sought from Congress for the second phase of the Safeguard (last year's bill for getting started on Safeguard was \$1,600 million) are requests for \$35 million for extra Sprint missiles which are now deemed necessary and for \$58 million for further design work on Project Hardsite.

Project Hardsite appears to be intended for just the purpose recommended by the O'Neill panel for Safeguard. It would defend fewer silos than the 150 protected by a Safeguard, each radar having a smaller range than the giant MSR and controlling fewer interceptors. The advantage of creating smaller units was explained to a House Subcommittee in April this year by Mr Richard L. Johnson, Assistant Secretary of the Army for Research and Development. He described how, by reducing the range of the system's radars, and hence the number of attacking missiles each can see, there are considerable savings in the size of the data processing problems involved. In other words, reducing the capability of the radar enhances its efficiency. Mr Johnson told the committee that the project was in the stage of "concept formulation", which meant deciding the optimum size of the radars and computers and showing analytically that it was a viable system. Dr Gilstein, head of the Pentagon's Advanced Ballistic Missile Defense Agency, added that the computers for Project Hardsite already

exist and the software is now being written. The technology for much of the radars also exists, through a complete radar has yet to be built. Asked about the likely cost of Project Hardsite (at the time of the committee's hearings Safeguard was being upheld as a way of protecting cities as well as missile sites), Mr Johnson said that it would be "less expensive than Safeguard to defend the Minuteman. If you are talking about the present amount for Safeguard it would be that or less."

What are the chances of either Safeguard or Hardsite being built? Proponents of the system, who have stressed its value as a bargaining chip, have put forward the comforting view that the Russians face a similar situation to that of the Americans, having a suspicious and obdurate military to deal with and even, it is implied, not so few bugs in the SS-9 that they wouldn't be as glad to ditch it as the United States would be to say farewell to Safeguard. One of the proposals announced at the end of the present SALT talks last week was in fact an American offer to limit Safeguard in exchange for a Soviet curb on the SS-9. The irony of the Cooper-Hart amendment which the Senate rejected is that in asking for a mere \$322 million reduction in the budget for Safeguard it would scarcely have diminished the credibility of the system or the present prospects for a trade-off against the SS-9. But it is little wonder that the Administration is as pleased as Punch with the defeat of the amendment. For if no bargain is struck the Administration will appear to have made a far sighted investment in a system vital for the nation's defence, and if Safeguard is traded away it will be Mr Nixon, not the Democrats in the Senate, who can claim credit for all the economies that will ensue upon its demise.

CYCLAMATES

FDA's Last Somersault

NEVER can an important agency of government have reversed on its stand so erratically or so frequently as has the Food and Drug Administration in the matter of cyclamates. In October last year, the Department of Health, Education and Welfare, on the FDA's advice, banned the artificial sweeteners in a manner that has served as the paradigm of how not to handle such matters. A month later the FDA partly reversed its stand by permitting some food and drinks containing cyclamates to be sold over the counter without prescription provided they were labelled as drugs. Last week the FDA reversed this decision too on the grounds that cyclamates are no longer effective as drugs.

It stretches the credulity near to its utmost limits that the "new information" on which the FDA has now acted became available in the form of an internal FDA memorandum dated November 26 last year, a bare seven days after the meeting at which the decision was taken to allow the sale of cyclamates as drugs. The memorandum, prepared by H. L. Richardson and colleagues, describes the feeding of cyclamates to rats for periods of about 100 weeks.

The basis of the new decision is the finding reported in the memorandum that the safe feeding level of cyclamates in rats is not 1,120 mg/kg a day, as appeared to be the case at the time of the original ban in October, but 240 mg/kg a day. Adjusting the dose downwards

to give the customary 100-fold safety factor, the assumed maximum safe daily dose of cyclamates is 2.4 mg/kg or 168 mg a day for a 70 kg adult. Since this is equivalent in sweetening power to only 5.1 g of sucrose or 21 calories, the benefit to a diabetic or weight-saver is negligible. Hence cyclamates are not effective as drugs, so that food products containing cyclamates will not be permitted to be marketed as such.

PSYCHOLOGY

Repeal of the Porn Laws

LORD CHESTERFIELD once remarked of sexual intercourse that "the pleasure is momentary, the position ridiculous and the expense damnable"; much the same set of emotions has been evoked in a congressional committee by the President's Commission on Obscenity and Pornography, which has had the misfortune to have a draft report of its findings leaked to the committee before being able to decide on its final recommendations. The research work performed for the commission (at a cost of some \$2 million over the past two years) indicates that the most harmful effect of exposing people to pornography, at least in a laboratory setting, is not so much a propensity to violent or deviant sexual behaviour as an insupportable boredom.

Other, perhaps less predictable conclusions of the draft report are that women no less than men may be excited by erotic films and pictures, that basic attitudes and patterns of sexual behaviour do not change after viewing pornographic films and, to give just an extra flutter to the red rag being waved before congressional noses, that the politically conservative are less easily aroused by pornography than those inclined to the left of the political spectrum. The draft recommendations—not yet accepted by the Commission—apparently call for laws that would be almost as liberal as those of Denmark, possibly the only country whose legal attitude to pornography is more tolerant than that of the United States.

The commission is expected to announce its findings on August 30 or later, but meanwhile the events occasioned by its draft report have taken a predictable turn. The Administration has been busily washing its hands of the Commission and all its doings, protesting that it was President Johnson who set up the Commission and appointed its members. And the House Subcommittee on Postal Operations, to which the draft report was leaked, has leapt in to quarrel with the Commission's conclusions and methodology. The committee's star witness last week was Dr Victor B. Cline, professor of psychology at the University of Utah, who in a statement to the commission in May had taken issue with the quip that "no girl was ever ruined by a book". "While this is slightly humorous, of course," Dr Cline admitted on that occasion, "we know this isn't really true. The Protestant Reformation was ignited by a written proclamation." No less firmly than Luther hammering his edict onto the church door at Wittenberg, Dr Cline banged the nails into what he hoped would be the Commission's coffin. He told the subcommittee that the report was "a gross mixture of truth and error, part science fiction and certainly a travesty as a scientific document attempting to do a judicious and fair survey of the literature in this area".

After reading the Commission's draft report (though without the original data) Dr Cline criticized its biased review of the literature, uncritical use of data and neglect of basic definitions. "To put it in a less charitable way, I doubt that any decent university psychology department would ever award a Master's thesis on the basis of such an incautious review of the literature as this is, and especially the unwarranted conclusions drawn from the data."

A hostile Congressional committee is not the cosiest forum before which to give the technical details of one's experiments their first public hearing, but this was the ordeal that faced two groups of psychologists who had undertaken research for the Commission. Dr Cline having prepared the way, it was the turn of Dr Sheldon Starr, of the Veterans Administration Hospital's Family Study Unit, to describe how he and his colleagues had investigated the effect of exposing married couples to pornography. Their subjects, all of whom had been married for at least ten years, were recruited from an advertisement in a Palo Alto newspaper (remarkably, 16 per cent of them had PhDs). The purpose of the study was to ascertain by questionnaires whether there was an alteration in the manner or frequency of the couples' conjugal relations after exposure to erotic films. The anticlimactic conclusion of the survey (which cost some \$20,000) was that "exposure to these films produced only a very brief and transitory increase in the subjects' sexual behaviour during the time the films were being shown. But in the period afterwards the level of sexual activity decreased."

Much the same tale was told by Dr James L. Howard, professor of psychiatry at the University of North Carolina. He and his colleagues exposed some 20 male students to pornographic materials for an hour and a half a day for three weeks, the subjects' response being monitored by a plethysmograph (an instrument for measuring small volume changes) and by assaying the quantity of acid phosphatase (an enzyme of the prostatic fluid) in urine samples. Although the subjects "thought more about sex" during the first few days of the experiment, there was no change in their previous patterns of sexual behaviour. To begin with, they spent about 90 per cent of their time looking at the pornography available for them but by the end of the experimental period only 35 per cent, and "there was a consistent downward trend all the way through the time course of the experiment".

Several of the subjects said they would refuse to participate in the experiment again because it was very dull, and Dr Howard thought the committee might be interested to know that "to a man they considered the question of the control of smut to be among the very minor social concerns of the day. Most of them found it difficult to think of a less important social issue."

ADDICTION

Programme on Heroin

from our New York Correspondent

IN an attempt to cut through the rhetoric and emotionalism that surround the heroin problem, Dr Gilbert Levin, an assistant professor of psychiatry at the Albert Einstein College of Medicine, has set a computer to work on the causes of heroin addiction in

a New York City neighbourhood. The study, supported by a \$100,000 grant from the National Institute of Mental Health, is meant to force administrators to think about the problem in logical rather than emotional terms. The computer model is constructed from a series of equations representing statements about the causes of developing problems in the area. The information has come from a variety of sources, including the police, addicts, parole officers, doctors and neighbourhood residents.

The area being studied contains approximately 180,000 people, most of them white. Dr Levin feels that the model will be applicable to a wide range of communities with a few alterations in the initial assumptions. "It would be reasonably easy to make the necessary changes except for the extremes—the very rich or the very poor," he said. With the help of Edward B. Roberts, a management professor at the Massachusetts Institute of Technology, and Gary Hirsch of Pugh-Roberts Inc., a Cambridge, Massachusetts, consulting firm, he has designed the systems analysis programme to provide a "time study" of how solutions applied to the problems would affect the situation over a 25 year period.

The study has not yet taken account of the British approach to legalized heroin although it intends to do so, but Dr Levin considers that in the United States, any extreme solution would have destructive short-run consequences. If the supply of heroin were suddenly increased through legalization, addicts could obtain heroin but the black market supply would have to find new users. This would lead to a selling drive among the young and the number of new addicts would increase. Equally, if the heroin supply were cut off suddenly, there would be an increase in crime as desperate addicts searched for enough money to buy the limited amount of heroin available. If either course were carried out more gradually, the results might be beneficial, Dr Levin said, but this could only be determined after more study.

The use of methadone, a synthetic drug that is addicting but is supposed to block both the effect of heroin and the addict's craving for it, allowing him to function normally, is on the increase in New York and throughout the country. It is not meant as a universal panacea, but too many communities see it as a simple solution to this complex problem. The computer suggests that if methadone alone were used, new addicts would be spawned faster than any methadone programme could handle them and that many younger addicts would reject it. Dr Levin is hopeful that methadone will prove a useful tool in combating addiction as long as it is not relied upon totally.

This is also true of the other stop-gap measures that have been tried in the past. The usual punitive approaches to addiction, treating it as a crime rather than the disease it is, have little if any effect in themselves. If the police alone are used to handle addicts, the computer predicted that the gaols and courts would be filled to overflowing with addicts and the neighbourhood would deteriorate, with middle-class families fleeing addict-related crime. "While the police are necessary to keep down drug availability within the community, thereby slowing the growth of addiction in the area, inroads into drug growth can only be made when the police are working simultaneously with other preventive approaches," Dr Levin said.

Potentially, education is one of the most effective ways to combat the growth of drug addiction, but no one is yet sure what programmes are really effective in reaching the young. Dr Levin makes the distinction between "credible" and "incredible" education. Incredible education includes some statements or attitudes that are false or exaggerated; such programmes have zero or negative effectiveness since all the information, both false and true, is rejected as untrustworthy. But if education can be credible, Dr Levin is hopeful that it can be effective.

MOON

Apollo Programme may be Docked

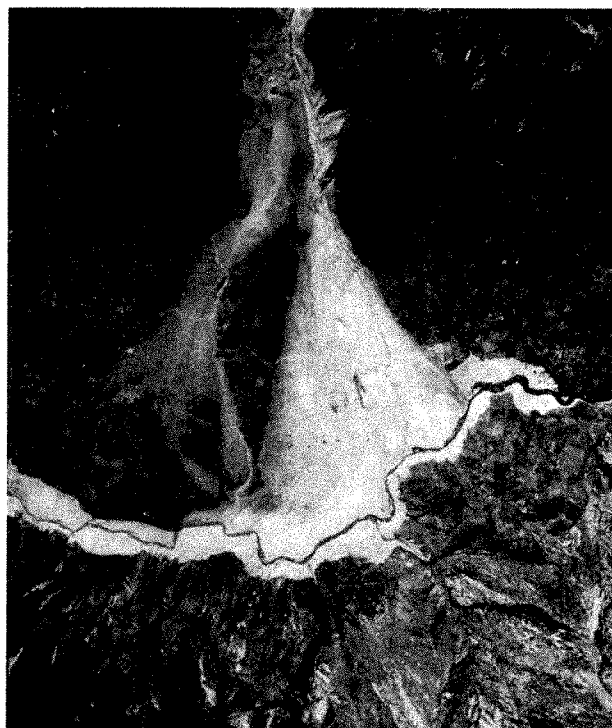
SELENOLOGISTS who felt that the Moon was almost within their grasp may have bad news in store for them next week when the future of the Apollo programme is decided. Although the number of scientifically important sites on the Moon is well in excess of the six Apollo flights that now remain, the National Aeronautics and Space Administration has long been toying with the idea of cancelling two of the flights so as to conserve the Saturn V launcher vehicles for ambitions nearer home, such as Skylab and the space station project.

The decision on whether to cancel the two Apollos is being weighed up by the Space Science Board of the National Academy of Sciences as well as by NASA's own Lunar and Planetary Missions Board. The two boards will report next week to a NASA management group which will then decide the future of the programme. Now that production of the Saturn V rocket has ceased, NASA has only seven vehicles left with which to continue the Apollo programme and to launch a Skylab, in which the third stage of a Saturn V would be fitted out as a workshop where astronauts would live and undertake experiments in Earth orbit. In these straitened circumstances, and without a back-up for the Skylab project, the chances of retaining six Saturn V rockets for the lunar landings have always seemed poor in view of NASA's need to be seen to be conquering new worlds. Although eventually the production of Saturn V rockets must be resumed if NASA is to live, the short term advantage of cancelling two Apollo flights will be to free a second Saturn for the Skylab and to keep one in reserve for launching a predecessor of the large manned space station that is planned for the late 1970s.

Deleting another two Apollo flights—the projected Apollo 20 has already fallen by the wayside—is likely to cause some distress and discouragement to lunar geologists who may find their researches left hanging in the air with crucial questions unanswered if the exploration programme is stopped too soon. A site in the lunar highlands has yet to be visited and even when that priority is fulfilled no selenologist will feel his appetite sated until a mascon basin has been inspected together with other unique features such as sinuous rills and Maar-type craters.

The remaining Apollo flights are of two types, known as H and J missions, of which the latter are equipped to allow for longer sojourns on the Moon's surface. If the decision is taken to curtail the programme, the last flight of each type, Apollos 15 and 19, will be dropped. Apart from the priority of visiting the Fra

Mauro highlands site, where Apollo 14 is scheduled to land in February, the remaining sites have not been decided because of the uncertainty that has prevailed about the programme's extent. With Apollo 14 already allocated, lunar geologists may soon face the invidious problem of how to maximize their knowledge of the Moon with only three further shots. Like the girl in the fairy tale, they will doubtless ponder carefully before spending their three wishes.



Peruvian earthquake—the photograph, taken by a NASA aircraft to help assess the extent of the damage, shows the mound of silt that buried the town of Yungay and 20,000 people. A slab of granite, dislodged by the earthquake from the Nevado de Huascaran, picked up glacial silt equal to about three times its volume and sailed down the canyon on a cushion of air. Most of the avalanche swept into the Santa River, but one tributary (to the left of the main flow) leapt over a 600 foot ridge and through the town of Yungay.

Miscellaneous Intelligence

Overheard in the Daddario Committee Room:

HANDLER: Chairman Miller, Mr Daddario, members of the committee, I am somewhat embarrassed in the sense that this very week there is an editorial in *Nature* magazine saying that I am the only scientist in Washington who can give a speech without notes.

I come before you with a rather lengthy statement. With that apology I will try to get on as rapidly as possible.

DADDARIO: We will try to keep it off the record so *Nature* magazine won't know about it.

HANDLER: They will know about it this afternoon.

NEWS AND VIEWS

Satellite DNA Sequence

WHAT possible function can be served by a DNA which consists of tandem duplication of a sequence of only six base pairs, and why should an animal such as the guinea-pig require some 10^7 copies of this short sequence in all its cells? Those are two of the intriguing questions which arise from the analysis of the base sequence of guinea-pig satellite DNA reported by Dr E. M. Southern on page 794.

Since satellite DNA was discovered some ten years ago, it has become increasingly clear that this fraction of DNA has remarkably curious properties (*Nature*, **225**, 899; 1970). It differs in buoyant density from the bulk of the cell DNA but, more importantly, the two strands of satellite DNA that are separated by heating reassociate very much faster than the rest of the cell DNA. This characteristic property of rapid renaturation implies that satellite DNA is highly reiterated and comprises a series of repeats of a short sequence. The length of the repeated sequence can be estimated from measurements of the rate of renaturation and on this basis it seems that mouse satellite DNA, which accounts for no less than 10 per cent of the total DNA in mouse cells, consists of multiple copies of a sequence of about 300 base pairs. By the same criteria the repeated sequence of guinea-pig satellite DNA has been estimated as 10^5 base pairs.

There are of course many pitfalls in making such estimates from renaturation data. Southern's chemical analysis of base sequences of guinea-pig and mouse satellite DNA proves how wildly inaccurate they can be. He finds that the basic sequence of guinea-pig satellite DNA is only six base pairs long and the sequence of mouse satellite DNA is somewhat longer—between eight and thirteen base pairs.

At present there are no enzymes for the cleavage of DNA which are as specific as the various ribonucleases used to cleave RNA. As a result the analysis of the sequences of DNA lags far behind comparable analyses of RNA. If, however, the two strands of a DNA can be separated, it is possible to derive the sequence for the whole molecule because reaction with diphenylamine degrades purines and liberates tracts containing only pyrimidines. Because base pairing occurs only between purines and pyrimidines the complete sequence of a DNA can be deduced from the patterns of pyrimidine tracts obtained when the two complementary strands of DNA are analysed separately. Fortunately some satellite DNAs are among the few species of DNAs which can be analysed in this way, because the complementary strands can be separated by sedimentation in alkaline caesium chloride gradients.

Using this approach, Southern finds that the basic

repeating sequence in guinea-pig satellite DNA is C-C-C-T-A-A in one strand and G-G-G-A-T-T in its complement. But if this satellite DNA consists purely of repeats of this extremely simple sequence, why do the kinetics of its renaturation suggest that the sequence is longer by several orders of magnitude? Southern believes that the answer is simply that during the course of evolution point mutations accumulated in the millions of copies of this short sequence. For example, the strand containing C-C-C-T-A-A has about 4 per cent G even though the basic repeating unit contains none of this nucleotide. Such mutations tend to mask the basic repeating unit and of course they also decrease the rate of renaturation of the two strands of satellite DNA. Estimates of the length of the repeating sequences obtained from the kinetics of renaturation are therefore bound to be overestimates. According to Southern's calculations, to produce guinea-pig satellite DNA the six-base sequence must have been reduplicated, either in one step or gradually, 10^7 times and more than 10^6 point mutations have accumulated. At first sight this seems an extremely large number of mutations, but the estimate is compatible with acceptable estimates of the mutation rate and the evolutionary age of satellite DNA.

What are the implications of this work? First, it is clear that satellite DNA is unlikely to code for any protein. It is inconceivable that the cell should need a protein that is simply a series of repeating dipeptides and it is interesting that of the six possible reading frames for satellite DNA two give alternating nonsense or chain terminating codons. Second, satellite DNA cannot have been subject to a master and slave mechanism because 10^6 mutations have accumulated in what, on an evolutionary scale, is a very short time. Finally, even though we now know the basic sequence unit of a satellite DNA we are no closer to explaining the function of these specialized DNAs. Since they have no role in coding protein the most plausible suggestion is that they have some role in maintaining the integrity of the chromosome itself. The localization of satellite DNA in the centromere regions of chromosomes (*Nature*, **225**, 899; 1970) suggests they play a part in the functions conventionally ascribed to the centromere. But for the time being such suggestions remain speculative. If, however, satellite DNAs really do have some centromeric function they should be present in all eukaryotes, although not necessarily in the large amounts found in some rodents. And Skinner, Beattie, Kerr and Graham (see page 837) report the presence of satellite DNAs, which have greater and smaller buoyant densities than the bulk of the DNA, in five species of crustaceans.

Could Palaeomagnetism be Wrong?

It is now clear that palaeomagnetic data provide the crucial evidence in favour of continental drift, sea floor spreading and plate tectonics, and the other ingredients of what has been called the "new global tectonics" in which the oceans are not only the youngest part of the Earth but are still being formed. The idea of global mobility has become the central dogma of Earth science. Naturally enough, like most dogmas it has attracted uncritical adherents.

Palaeomagnetic data are not the only evidence for global mobility, but they have the advantage of being quantitative. Although palaeontological, geological and palaeoclimatological data are mostly consistent with continental movements defined by palaeomagnetic poles, they usually have the status of supporting evidence simply because they are more difficult to quantify. Thus the doctrine of continental drift is consistent with what is known of the Earth's climatic zone pattern, but the broadness of the climatic zones means that it has been difficult to make precise use of climatological criteria. It has therefore always been an open question as to what would happen if anyone were to compare the results of palaeomagnetism more quantitatively with evidence from other sources.

This crunch has now come, although in a small way. The story is this. Continental movements are determined palaeomagnetically on the assumption that throughout the period covered by the rock record, the Earth's magnetic field has been axially dipolar. Needless to say, this is a reasonable assumption not internally inconsistent, but equally not susceptible to direct proof. Yet without such proof, palaeomagnetism falls to the ground. But Stehli (*J. Geophys. Res.*, **75**, 3325; 1970) now claims, on the basis of an analysis of palaeontological data, that for the Permian the axial hypothesis is not valid. Taken in isolation, his evidence is convincing because it is quantitative. His analysis is based on the observation that certain brachiopods are temperature dependent. The lower the temperature at any given place, the fewer brachiopod families there will be, which means that the diversity of brachiopod families will be a maximum around the equator but will fall off as the latitude increases. That this is the case is shown by the behaviour of Recent clams whose family diversity falls off quadratically with increasing latitude. The problem with Permian brachiopods from the northern hemisphere is that family diversity does not vary quadratically with palaeomagnetic latitude but does so when plotted on the present latitude grid. When plotted on the Permian palaeomagnetic latitude grid the Permian diversity bears no particular relationship to latitude, just as when the Recent clam diversity is plotted on the Permian palaeolatitude grid there is, as would be expected, no simple relationship. In short, the Permian axial field is not consistent with the Permian brachiopod diversity whereas the present latitude grid is. And if this is true for the Permian it could be true for other

geological periods. That is why palaeomagnetists cannot afford to ignore Stehli's conclusions.

The first and most obvious question to ask, of course, is whether the palaeontological sampling was adequate, for clearly the number of brachiopod families detected at a given latitude will be critically dependent on the intensity of sampling. Stehli, at least, is satisfied on this score. In the samples of Recent clams there were sixteen families of "cosmopolitan dominants" which, being more or less temperature independent, were found at more than 70 per cent of the sampling stations. These were used to test the sampling efficiency. On the assumption that perfect sampling would reveal all sixteen families, the number actually discovered at any given sampling station gives a crude indication of the total proportion of families sampled. The cosmopolitan dominants themselves were not included in the diversity calculations simply because they were not temperature dependent and thus of no use in testing latitude dependence.

The second question which can legitimately be asked is whether the palaeomagnetic data from North America and Eurasia are sufficient to define the true geomagnetic field for the Permian. The answer is that they are, as far as it is possible to tell, because the North American and Eurasian poles agree when the two continents are reconstructed in their pre-continental drift positions determined without reference to palaeomagnetism. This is in itself evidence—but not proof—in favour of the Permian axial dipole field because pole positions are calculated using the axial dipole assumption.

If Stehli's analysis is valid—and it is difficult at present to show that it is not—the only conclusion to be drawn in isolation is that the Permian field was not axial. Palaeomagnetic evidence, on the other hand, cannot prove that it was axial but does offer strong evidence to that effect. Palaeontological and palaeomagnetic evidence are thus in conflict, which means, unless some unifying principle is discovered, that one or the other is wrong.

ELECTRON BEAMS

Let There be Light

by our Solid State Physics Correspondent

Two conflicting explanations have now appeared to account for the experiment of Schwarz and Hora in which a beam of electrons was found to be imprinted with the frequency of an impinging laser beam (see *Nature*, **225**, 15; 1970). B. M. Oliver and L. S. Cutler (*Phys. Rev. Lett.*, **25**, 273; 1970) attribute this behaviour to a bunching of the electrons, but L. D. Favro, D. M. Fradkin and P. K. Kuo (*Phys. Rev. Lett.*, **25**, 202; 1970) suggest that the role of the laser beam is to modulate the wave-function of the electrons.

The original experiment (*Appl. Phys. Lett.*, **15**, 349; 1970) has created a great deal of interest. A beam of electrons was passed through a thin crystalline sheet

along which a laser beam was shone. If the laser light is polarized parallel to the electron beam, the frequency of the light is picked up by the electrons and is emitted again when the beam strikes a non-luminescent screen. Apart from the need to account for the mechanism of transfer of the frequency, the experiment may yet have implications for the design of television systems.

Oliver and Cutler suggest that the role of the thin sheet is to provide a short and sharply bounded region in which the electric field is reduced because of the dielectric property of the solid. The crystalline structure of the solid produces Laue spots. Variations of the electric field associated with the laser beam then produce sinusoidal fluctuations in the velocity of the electrons which are equivalent to a bunching of the particles. The effect of the sheet is to reduce the electric field to a level at which the interaction becomes observable. On this view, when the laser radiation is finally emitted at the screen, it should be polarized in a plane determined by the wave-vector of the bunched electrons. This point has not yet been tested experimentally.

Favro, Fradkin and Kuo claim that for usual laser intensities, the velocity imparted by the laser field is too small compared with the spread in velocities of the beam for bunching to take place. They suppose that the light actually modulates the wave-function of the electron, producing an effect which should be observable in synchrotron radiation, bremsstrahlung and Čerenkov radiation.

Both explanations avoid a proper quantum mechanical treatment, the latter being based on the assumption of an electric field created by point particles with unit charge. Oliver and Cutler point out, on the other hand, that although their analysis in terms of bunching seems quite reasonable the first indications of a quantum mechanical treatment suggest that genuine quantum phenomena are present.

ATOMIC ENERGY

Power and the Environment

from a Correspondent

THE availability of power is one of the greatest factors influencing the extent to which man has modified his environment. Less obviously, it is also the key to the ability to reduce environmental pollutants. At the start of the IAEA symposium on environmental aspects of nuclear power stations held in New York from August 10 to 14, Dr M. K. Hubbert (US Geological Survey) showed how rapidly the increasing world population is consuming its power resources. This theme recurred in a symposium principally devoted to the direct impact of power generation on the environment.

A session devoted to the setting and use of standards was chiefly concerned with basic radiation standards, which seemed to be better established than others, as well as being internationally accepted. These standards relate principally to radiation dose to man, and a contribution from Dr A. Preston (Fisheries Radiobiological Laboratory, UK) was typical of many that showed how the standards can be used to derive discharge limits for waste. Widespread use was reported of the technique of estimating the dose to the most highly exposed group of people (often called

the critical group) through the most important route in the environment, which is often a foodstuff. It emerged that detailed procedures had wide variations, chiefly reflecting different national regulatory practices. Reviewing the basic radiation standards, both Drs E. E. Pochin (University College Hospital Medical School, London) and K. Z. Morgan (Oak Ridge National Laboratory) emphasized the caution inherent in the setting of these standards, resulting principally from the assumption of a linear relationship between risk and dose irrespective of dose rate. Standards for air pollution and thermal effects in rivers and estuaries were also discussed but in a much less definitive way.

A series of sessions on the control and monitoring of effluent proved repetitious, although participants concerned with thermal effects claimed to be well satisfied. Outside Britain, monitoring programmes still seem to be proliferating without adequate attention to objectives, and a suggestion by Mr H. J. Dunster (UK Atomic Energy Authority) that monitoring round nuclear installations could be reduced after a few years of operating experience was regarded, especially by delegates from the United States, as interesting but unrealistic.

Contributions concerned with the siting of both nuclear and fossil fuelled power plants were more qualitative than is usual in this highly technical area, and several stressed the importance of gaining public and regulatory acceptance for urban siting. Attempts to bring different types of risk into perspective culminated in contributions from Drs C. Starr (University of California) and F. D. Sowby (International Commission on Radiological Protection). Both gave quantitative estimates of conventional risks which people accept, and suggested ways in which comparisons might be made with the possible radiation risks. These comparisons were difficult, but indicated that radiation risks were much smaller than other risks, such as the 3 per cent chance of being killed at work, to which air crews of scheduled airlines are exposed. Starr also attempted a link to the public awareness of the benefits associated with the risks.

At the end of the final session, on prospects for the future, Dunster welcomed the recent upsurge of interest in environmental matters, an interest which, he said, the power generating industry had been evincing for several decades. He concluded that the symposium had shown that people could be provided with "clean power in sufficient amounts for them to tackle the enormously difficult and pressing problems of man's survival as an advanced social species".

PHOTORECEPTORS

Improving on Nature

from our Molecular Biology Correspondent

ONCE in a while it becomes too great a strain for molecular biologists to maintain their posture of humble seekers after truth, and they give way to the impulse to jolly nature along a little. (Come to that, and mentioning no names, recent developments in molecular biology leave one with the inescapable impression that certain of its mandarins believe themselves to have designed it all in the first place.) An attempt to devise a photoregulation system such as nature might have evolved is described by Bieth, Wassermann, Vratsanos and Erlanger (*Proc. US Nat.*

Acad. Sci., **66**, 850; 1970), and if the philosophy of this approach is perhaps a little like designing the internal combustion engine in order to find out how muscles work, the result is nevertheless a diverting and technically accomplished exercise.

In earlier work from Erlanger's laboratory, enzyme inhibitors were synthesized which were able to undergo reversible photochemical changes to forms of differing activity. One such was an acetylcholine analogue, which could be introduced into electroplax cells from the electric eel, thereby rendering the system photosensitive. A transition from a state of strong to one of weaker activity was elicited by irradiation in the visible, and the reverse process by exposure to ultraviolet. The task, however, that Erlanger and his colleagues set themselves was to achieve a system which could be regulated by sunlight and darkness, and would thus mimic diurnal processes of nature. Success has now crowned their efforts. The inhibitor—again of acetylcholinesterase—is a carbamylcholine function substituted in one of the phenyl rings of azobenzene ($\varphi-N=N-\varphi$), a molecule which is subject to chemically induced *cis-trans* isomerization about the $N=N$ bond. The inhibitor binding constants of the two forms differ by a factor of rather more than two, in favour of the *cis* isomer. The *trans* isomer has an absorption band in the near ultraviolet, where the intensity of bright sunlight is sufficient to cause immediate conversion to a 50 per cent mixture of isomers. (This is New York City at midday in August of last year.) In the dark, or for that matter in the artificially lit laboratory, almost complete reversion to the stable *trans* form rapidly ensues. In these conditions, changes of some 10 per cent in activity of the acetylcholinesterase were triggered by exposure to sunlight, or 30 per cent if an ultraviolet source was used to drive the isomerization to completion. More efficient photoisomerization seems to be possible with a halogenated derivative; but as Bieth *et al.* disarmingly say, they have to await "better climatic conditions" before testing the new system with sunlight, being no doubt consoled during their vigil by the reflexion that the man-made filter of carbon monoxide, sulphur dioxide and tetraethyl lead would have defected nature herself. Bieth *et al.* conjecture that the carotenoids of animals and plants, which as all the world knows are prone to *cis-trans* isomerism, might function as photoregulators, controlling diurnal and seasonal changes in metabolic levels.

Vision is, of course, a system with obvious parallels, for it is triggered by isomerization of the carotenoid, retinene. To bind to the protein, opsin, all-*trans* retinene must isomerize to the bent 11-*cis* form, though the unnatural 9-*cis* isomer will also bind, but not 13-*cis*. One of the curious features of the photoisomerization is the preferential formation of the 11-*cis* form, in spite of crowding at the *cis* bend, caused by the methyl group on the thirteenth carbon in the chain, and the hydrogen on the tenth, which must force the chain into a twisted shape. In the continuing attempt to define the features of the carotenoid that determine its interaction with the opsin, Nelson, deRiel and Kropf (*ibid.*, 531) have synthesized a retinene lacking the 13-methyl group. It turns out that both the 11-*cis* and 9-*cis* isomers of this analogue associate with opsin, but the 11-*cis*, unlike its natural counterpart, binds only weakly, with a sizable proportion of free ligand and protein at equilibrium. It

bleaches normally, however, to the all-*trans* state, though at a very slow rate, presumed to arise from the lower strain energy of the bound *cis* isomer in the absence of the 13-methyl group. Either the difference in shape or the absence of the methyl group *per se* evidently causes the analogue to interact more weakly with the protein. It is perhaps more surprising that it should fit at all. It is known from earlier work that removal of the 9-methyl group likewise leaves a product of which the 11-*cis* form will bind to opsin.

COFFEE

Streamlining the Harvest

by our Botany Correspondent

A COMPOUND which has been attracting attention from plant physiologists for its effects on the ripening and abscission of fruit could have considerable practical value for cultivators of coffee. Trials of 2-chloroethane phosphonic acid (CEPA) on *Coffea arabica* in Kenya (G. Browning and M. G. R. Cannell, *Journal of Horticultural Science*, **45**, 223; 1970) suggest that there is valuable potential for controlling the time of ripening and the thinning of unwanted fruit.

One of the reasons for interest in CEPA at the Coffee Research Station at Ruiru in Kenya is that the cost of picking high quality coffee by hand could be reduced if the length of the ripening period could be influenced. In East Africa, small amounts of coffee sometimes have to be picked from all trees each month, both because the regime of light and dark is continually conducive to the induction of flowering, and because the ripening of fruit from a single flowering is usually spread over several weeks. The advantage of concentrating ripening into a few peak periods to allow for more efficient use of labour is clear.

A further reason for experimenting with CEPA is that it might often be useful to be able to control the abscission of fruit. If, for example, unwanted flowerings could be eliminated, trees might be induced to produce fruit when climatic conditions are most suitable for the development of berries and, subsequently, high quality beans.

As far as abscission is concerned, a single spraying of 1,400–1,500 p.p.m. of CEPA caused 60–65 per cent of young berries to drop at a time when they were expanding rapidly. In a concentration of 1,400 p.p.m. CEPA is not unduly toxic, although up to 10 per cent of leaves may senesce and fall from the trees. CEPA had little or no effect on flowers and berries at any other stage of development, nor did it promote the abscission of ripe fruit. Thus, although it might be possible to use this compound for thinning young fruit, it would not seem to be a potential aid to mechanical harvesting.

There are also promising signs that ripening could usefully be controlled with CEPA, which seems to act by liberating ethylene, the effects of which are well known. In a concentration of 700 or 1,400 p.p.m., a single spraying of CEPA accelerated the onset of ripening, so that the principal period of picking was brought forward by 2–4 weeks. Another good sign was that more ripe fruit was picked from trees sprayed with CEPA than from control trees. There was no shortening of the period of harvest on treated trees, nor was the quality of the coffee beans affected. It

seems likely that CEPA could be used to synchronize the ripening of fruit from flowers which develop a few weeks apart so that picking could be concentrated into fewer periods. Clearly, if more trials prove successful, Kenya's coffee industry could benefit considerably from the use of this compound.

MICROBIOLOGY

Warnings and Advances

from a Correspondent

A NEW awareness of the social responsibilities of microbiologists seemed to pervade the atmosphere of the international congress of microbiology which began in Mexico City on August 9. Setting the scene for the 4,000 delegates, Dr Andre Lwoff (Pasteur Institute, Paris) dwelt on the need for microbiologists to ensure that their efforts are not diverted into such uses as microbiological warfare. He defined their goal as the improved health of mankind, and called on participants to publicize this ideal and remind their governments of the dangers of germ warfare. His point was taken up by the local newspapers which, in giving prominence to the congress, reflected an awareness of the difference between the potential and actual achievements of scientists. Another warning came from Dr S. E. Luria (Massachusetts Institute of Technology) who stressed the powers of microbiologists who can manipulate single genes. It is now practical, he said, to contemplate the possibility of altering the genetic constitution of any kind of living organism, with incalculable consequences.

On a more practical note, Dr Rodrigues-Leiva

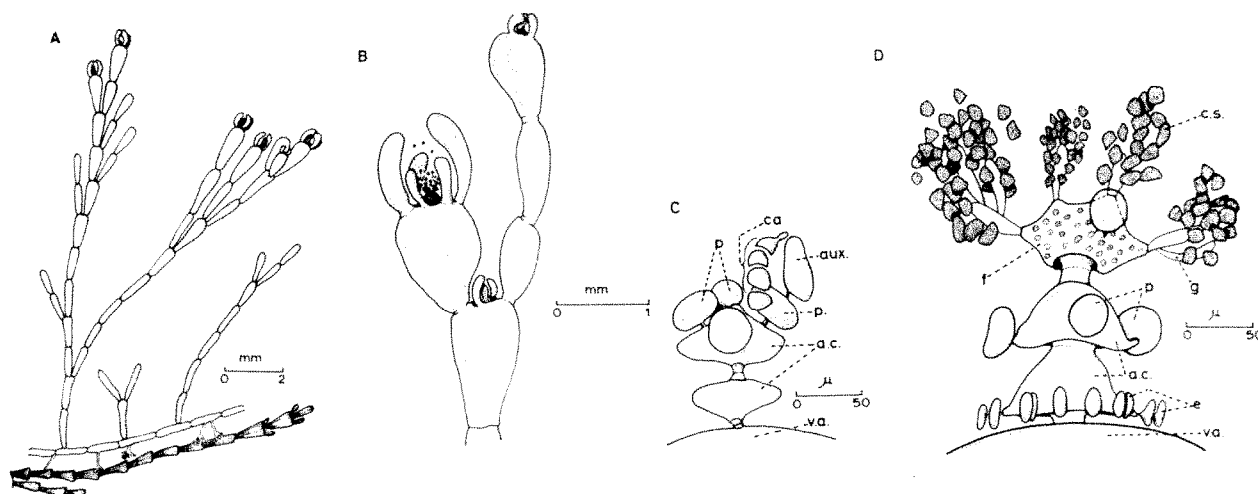
(Chile) pointed out the difficulties that face practitioners of preventive medicine in countries such as those of South America, which have to import all their supplies of vaccines. If adequate quantities of vaccines against poliomyelitis and other prevalent childhood infections could be produced locally, these illnesses could be wiped out easily. But they remain prevalent because of the high costs of importation.

There was more interest for delegates from developing countries in a symposium on single cell protein. They learnt that by growing various bacteria or fungi on readily available substrates, such as cellulose or hydrocarbons, enough first class protein could be manufactured to make a significant contribution to the food supply for man and animals. Several industrial techniques were described, and it was evident that the social and political implications would require vigilance if this new application to human welfare was to be fully exploited.

The principal medical themes of the congress were antibiotics and immunity. Although new antibacterial and antifungal antibiotics were discussed, there does not yet seem to have been a breakthrough on the antiviral front. There are, however, few bacterial or fungal disorders which cannot be controlled by antibiotics. One such group of disorders consists of the infections caused by *Pseudomonas* and similar Gram negative bacilli. Antibiotics are still of limited value in the treatment of these cases, and there is a new awareness of the possible value of active and passive immunization. Several speakers stressed the importance of antibody response in *Pseudomonas* infections and the possibility that it could be enhanced by active immunity.

ALGAE

New Species from the Sea



The shores of southern Africa have yielded six new species of seaweed; one brown and five red algae have been described by R. H. Simons (Division of Sea Fisheries Investigational Report No. 86, Cape Town, 1970). *Griffithsia cymosa*, the red alga, which is shown here, produced a furry growth on a sheet of 'Perspex', which was covered with anticorrosive paint and immersed in the sea in an attempt to discover the sort of organisms that foul the bottoms of ships. The other species described by Simons include *Digenea subarticulata*, which belongs to a genus formerly thought to be monospecific, and *Digeneopsis subopaca*, the first member of a newly founded genus. The brown alga, *Cystophora fibrosa*, was previously thought to be confined to Australasian waters.

In the illustration of *Griffithsia cymosa*, A shows the general habit of the plant; B is the cymose arrangement of cystocarpic segments; C is a fertile dwarf shoot at the apex of a vegetative branch, and D is a stage in the development of the fertile dwarf shoot after fertilization. a, Axial cell of dwarf shoot; ac, auxiliary cell; c, carpogonial branch; cs, carpospore; e, enveloping threads which remain vestigial; f, fusion cell; g, gonimoblast filament; p, pericentral cell; va, vegetative axial cell.

Explosive Nucleosynthesis in Stars

by

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Recent calculations provide convincing evidence that the naturally occurring nuclei were produced in explosions. The required temperature, density and expansion rate strongly suggest that before the explosion the objects were ordinary evolved massive stars. We review these new developments and present a new table indicating our hypothesis concerning the origin of the nuclei in the mass range $20 \leq A \leq 62$.

IN 1957, Burbidge, Burbidge, Fowler and Hoyle set the stage for at least two decades of research into the origin of the elements. They expressed¹ the opinion that the requirements for a proper theory "are mainly satisfied by the fourth theory in which it is proposed that the stars are the seat of origin of the elements. In contrast with the other theories which demand matter in a particular primordial state for which we have no evidence, this latter theory is intimately related to the known fact that nuclear transmutations are currently taking place inside stars".

In the following year Schwarzschild's monograph, *Structure and Evolution of the Stars*, systematically discussed the exciting progress in understanding static stars, which astronomers had long observed to be virtually unchanging. For the computational problem he observed²: "It is this constancy of stars which makes a definite analysis of the stellar interior possible. It asserts that the stellar interior must be in perfect equilibrium and thus limits the necessary analysis to equilibrium configurations". It was natural then that nuclear physicists interested in the thermonuclear origin of the elements should turn their attention to the details of the thermonuclear reactions that occur in the static nuclear burning stages of the evolution of the stellar interior. Not only did these studies fill in the details necessary for computation of the slow evolution of the star, but it was often tacitly assumed that the nuclear ashes of these epochs would somehow provide an understanding of the observed abundances of the elements.

In the past two years or so, the situation has changed markedly. In many respects the change was presaged by the discovery by Schwarzschild and Härm³ that the ignition of helium in the hydrogen-exhausted core of a low mass star occurs in the presence of a degenerate electron gas which is providing the bulk of the pressure support. Because the pressure of such a gas does not increase substantially when the temperature rises whereas the rates of thermonuclear reaction increase dramatically with increasing temperature, a brief runaway in the thermonuclear activity ensues (the helium flash). After the degeneracy is removed by heat input, the star in this case quickly expands after only a small degree of nuclear burning to an adjusted configuration where burning can proceed in hydrostatic equilibrium. With the subsequent discovery of the very effective cooling of more evolved

stellar interiors due to neutrino emission, it has become apparent that intrinsically more explosive nuclear fuels, namely, ^{12}C and ^{16}O , may also ignite in a very degenerate electron gas and that in this case the runaway in nuclear reactions may be great enough completely to disrupt the star via a thermonuclear explosion^{4,5}. The high temperature of the explosion, which lasts only a fraction of a second, produces such a high degree of nuclear processing that the expelled thermonuclear products are vastly different than the composition of the mass zones before the explosion^{5,6}. The main point we wish to make in this article is that the natural abundances of the elements provide convincing testimony to the fact that they were indeed synthesized in just such events.

The idea that the bulk of the newly created elements were ejected from supernovae is certainly not new; indeed, important papers^{1,7} gave prominent attention to supernovae as a chief source of new matter for the interstellar medium. The recent dramatic progress stems from the realization that the composition of the entire star probably changes in a few explosive terminal seconds and from the development of accurate numerical computation programs for the network of nuclear reactions that proceeds during the explosion. The results of these explosive chains of nuclear reactions can now be seen to be virtually identical to the abundances of the elements and their several isotopes.

Simulation of the Event

To follow realistically the results of nuclear burning in an exploding star we need three key types of information: (1) an understanding of the mechanism of the explosion and the time history of the temperature and density of the exploding matter during the ensuing expansion; (2) knowledge of the composition and state of matter before the explosion; and (3) understanding of the rates of large numbers of thermonuclear reactions. Much experimental and theoretical work remains to be done on the cross-sections of thermonuclear reaction, but due largely to the work of Fowler and his colleagues⁸ on assembling and analysing nuclear data and to the work of Cameron and his colleagues⁹ on the statistical evaluation of thermonuclear cross-sections for the interaction of protons, neutrons, alpha particles and γ -rays with target nuclei primarily in the range $20 < A < 60$, we have estimates of cross-sections which are adequate for the task of

surveying the likely results of explosive burning of nuclear fuels of intermediate mass. With these rates Truran and Arnett and their colleagues have constructed numerical reaction networks appropriate to the explosive conditions and fuels.

The key thermonuclear feature of explosive burning is that the several fuels combust (in the nuclear sense) at temperatures considerably higher than those at which the same fuels burn in an object in hydrostatic equilibrium, with considerable effect on the abundances of the ejected matter. The overheating may result either from the fact that the fuels first ignite in a degenerate electron gas or, for the non-central mass zones, from the compressional heating produced as a strong pressure wave propagates outward from an exploding core. In either case, large amounts of thermal energy are liberated in a time short compared with the star's ability to compensate hydrodynamically, with the result that the entire star may be given positive energy sufficient to disrupt it. In either case the time scale for the expansion should be of the order of the hydrodynamic time scale for free fall or free expansion

$$\frac{1}{\rho} \left| \frac{d\rho}{dt} \right| \equiv \frac{1}{\tau_{HD}} \approx (24\pi G\rho)^{\frac{1}{2}} = \frac{1}{446 \rho^{-\frac{1}{2}}} \text{ s}^{-1} \quad (1)$$

where the mass density ρ is expressed in c.g.s. units. Although large amounts of energy are liberated initially the subsequent expansion should be nearly adiabatic

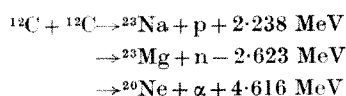
$$\rho T^{-3} \approx \text{constant} \quad (2)$$

The investigations so far have combined equations (1) and (2) with an estimate of the initial density and peak temperature of the explosion to yield an estimate of the thermal history of the expanding matter. The nuclear reaction codes then explicitly compute the nuclear abundances established during the explosion.

The initial composition and thermal conditions must be generated by computing the complete evolution of the star up to the point of explosion. When that is accomplished, the complete evolution through the explosion will also be done explicitly with hydrodynamic techniques, but for the time being the most dramatic results have been obtained by simulating the explosion with the aid of equations (1) and (2) and judicious guesses concerning the initial state.

Nuclear Evidence

The first indication of the importance of the dynamics of the expansion on the final nuclear products came in a study of carbon burning. Arnett and Truran¹⁰ established a numerical scheme for solving the nuclear reaction network that results when ^{12}C nuclei begin to undergo the fusion reactions



A large number of coupled reactions become possible as the liberated protons, neutrons, and alpha particles begin to react with all of the nuclear species generated within the gas; in fact, Arnett and Truran used rates for 99 different nuclear reactions in their carbon burning network. Despite this complexity, the results they obtained for the final abundances following carbon burning at a constant temperature near 10^9 K, a value at which carbon

would be expected to burn in the core of a star in hydrostatic equilibrium, were very simple. Only ^{20}Ne , ^{23}Na and ^{24}Mg were produced in substantially significant quantities, and approximately in the ratio of their abundances as observed in the solar system. Although this work confirmed that these three nuclear species almost certainly owe their high natural abundance to carbon burning in stars, it left the source of the other isotopes in this mass range a mystery.

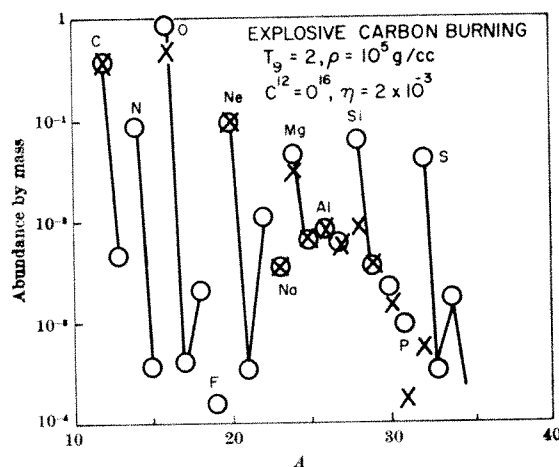


Fig. 1. Products of explosive carbon burning. Circles are used to represent solar system abundances by mass, solid lines connect all stable isotopes of the given element and calculated abundances are shown by crosses. The vertical axis is measured in arbitrary units of abundance by mass, X ; the horizontal axis in units of atomic number A . The peak temperature was 2.0×10^9 K, the peak density $\rho = 1.0 \times 10^5$ g cm⁻³ and the neutron excess was $\eta = 0.002$ (see text).

At this time it occurred to Arnett⁵ to make an important numerical experiment. He supposed that for one or a combination of the reasons mentioned earlier, the carbon could be forced to ignite explosively at temperatures near 2×10^9 K, which is far in excess of temperatures at which carbon naturally burns in a static object, and at initial densities in the range $10^5 < \rho(\text{g cm}^{-3}) < 10^9$. A coupling of equations (1) and (2) to the same nuclear reaction network resulted in dramatic changes in the final abundances. A typical result is shown in Fig. 1, where the open circles indicate Cameron's relative solar abundances¹¹ and the crosses represent the final abundances calculated with the reaction network. Before the explosion the gas was taken to be virtually half and half ^{12}C and ^{16}O as produced in a previous epoch of helium burning, plus 2 per cent of ^{18}O which is the result of the earlier conversion within the same star of all of the original CNO nuclei into ^{18}O by hydrogen burning and helium burning in turn. In the computation of Fig. 1 the carbon has burned furiously for about one-tenth of a second at which time reactions are frozen by the falling temperature associated with the vigorous expansion of the gas. Most of the carbon and virtually all of the initial oxygen remain unburned, such that the final ratio $^{12}\text{C}/^{24}\text{Mg}$ matches the solar ratio. More significantly, the nuclei ^{20}Ne , ^{23}Na , ^{24}Mg , ^{25}Mg , ^{26}Mg , ^{27}Al , ^{28}Si and perhaps ^{30}Si and some ^{31}P , are produced in ratios closely approximating the solar abundances. The fact that abundant ^{28}Si is unaccounted for is no weakness, because it is known to be a primary result of oxygen burning. The suggestion is strong, therefore, that these nuclei owe their existence to the explosive ejection of partially burned shells of carbon.

An especially important aspect of the explosive carbon burning is that the final abundances of nuclei having more

neutrons than protons (for example, ^{23}Na , $^{25,26}\text{Mg}$, ^{27}Al) depend on the degree of neutron enrichment initially present when the ^{12}C begins to burn, because there is inadequate time during explosive burning for beta decays to increase the neutron number per gram. The neutron excess is largely due to the isotopes of C, N and O present in the initial composition of the star, constituting roughly 2 per cent by mass in the Sun. Before carbon burning ignites, these isotopes will have been converted to ^{14}N during the CNO cycle and to ^{18}O during helium burning. There results a neutron excess

$$\eta \equiv \frac{n_n - n_p}{n_n + n_p} \quad (3)$$

which has the value $\eta \approx 0.002$ for a composition like the Sun. It is these excess neutrons, roughly 0.2 per cent by mass, that end up as the neutron rich products ^{23}Na , $^{25,26}\text{Mg}$ and ^{27}Al . We will return to this question later in discussing differences in nucleosynthesis between Population I stars and Population II stars. For the time being, we point out Fig. 2, which shows how the abundances of several elements (including specifically Na and Al) depend on the neutron enrichment prior to explosive ignition.

A significant thing happens to the heavier nuclei present in small traces when the carbon burns explosively. The burst of neutrons liberated by the primary burning causes a series of neutron reactions on the heavy seed elements, and we have shown that the rare neutron-rich

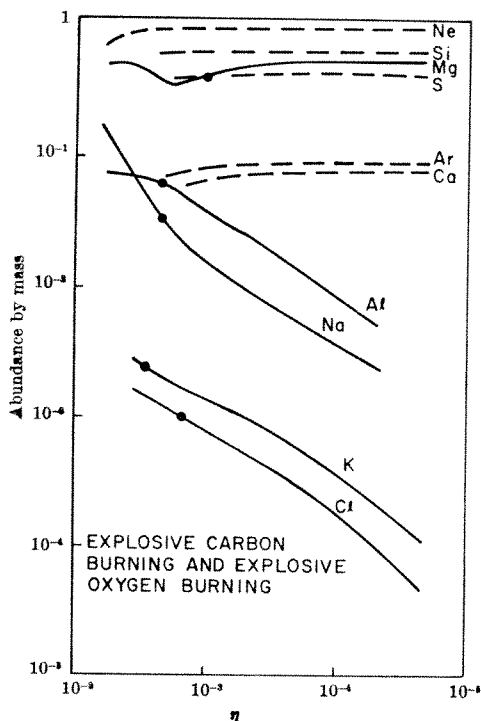


Fig. 2. Elemental abundances from explosive burning of ^{12}C and of ^{16}O as a function of neutron excess η (see text). The elements of whose most abundant nucleus has an alpha-particle structure are relatively insensitive to variation of η , and with the exception of magnesium they are represented by dashed lines. In the case of magnesium, which is represented by a solid line, the relative abundance of ^{25}Mg compared with $^{26,27}\text{Mg}$ does vary with η . Such an effect might be observable. A heavy solid circle indicates the point on the curve at which both the elemental and isotopic abundances for magnesium have their solar values. The odd- Z elements are also represented by solid lines. Solid circles indicate where their elemental abundances agree with solar values. Note that the solid circles cluster around $\eta \approx 0.002$. This phenomenon is simply explained if the explosive progenitor of the Sun were a Population I ("metal rich") object (see text).

nuclei ^{36}S , ^{40}A , ^{40}K , $^{46,48}\text{Ca}$, ^{50}Ti , ^{64}Ni , ^{66}Zn , ^{70}Zn and ^{72}Ge very likely owe their natural abundances to these neutron reactions (unpublished). This result has special significance because these are almost all of the major nuclei not produced during the explosive burning of oxygen or silicon. There is, in fact, considerable promise that the requirement for manufacturing these neutron-rich nuclei may delineate special features of the thermal history of the carbon burning.

If the peak temperature in carbon burning rises as high as $T_9 = 3$ the ^{16}O also will ignite explosively, thereby driving the temperature even higher. The primary reaction channels for reactions of $^{16}\text{O} + ^{16}\text{O}$ are $^{28}\text{Si} + ^4\text{He}$, $^{30}\text{P} + \text{d}$, $^{31}\text{P} + \text{p}$ and $^{31}\text{S} + \text{n}$, and the corresponding reaction network builds nuclei at $A \geq 28$. A typical result⁶ of the reaction network is shown in Fig. 3. Virtually all of the nuclei having $32 \leq A \leq 42$ are produced in their observed ratio to ^{28}Si in this particular expansion. Only the rare neutron-rich nuclei ^{36}S and ^{40}A are not adequately produced in that mass range; those two nuclei, however, have already been noted as produced by neutron reactions on S, A and Ca seed nuclei present in small amounts in explosive carbon burning. The nuclei ^{28}Si , ^{30}Si and ^{31}P are not adequately produced by this expansion, but other oxygen burning conditions can have a larger yield of those nuclei, and carbon burning also contributed substantially to their abundance. Thus the success of this calculation is so great that it seems that the ideas must be very close to the truth.

Just as in carbon burning, so it is also in oxygen burning that the element abundances of neutron-rich elements, especially Cl and K for calculations like that in Fig. 3, depend on the degree of neutron enrichment in the gas when the oxygen ignites. Selected results are shown in Fig. 2, where we again see that the neutron excess $\eta \approx 0.002$ is close to the value required for synthesis of the solar system abundances of Cl and K. The demand that the neutron enrichment is not too large seems (see Fig. 10 and Table 3 of ref. 10) to require that the oxygen burning mass zones which are explosively ejected have not been processed previously through hydrostatic carbon burning at $T_9 < 1$, or that the explosion is generated before the hydrostatic carbon burning is complete. In any case, we believe that the replication of known abundance ratios will be possible only for specific correct models of the nucleosynthesis event.

As the oxygen fuel is exhausted, oxygen burning merges continuously into silicon burning. Instead of undergoing fusion reactions, the silicon burns by a process called¹² "photodisintegration rearrangement". In this process the ^{28}Si is slowly (compared with other reaction lifetimes) photodisintegrated into seven alpha particles, which are in turn quickly captured by remaining ^{28}Si nuclei, thereby establishing a tail of higher atomic weight attached to ^{28}Si . Because the nuclei having $A > 28$ suffer photoejection reactions more rapidly than does ^{28}Si , however, the capture flow tends to be balanced by the inverse reaction. Bodansky, Clayton and Fowler¹³ and Truran¹⁴ showed that the nuclear system attempts, and usually succeeds, in establishing what they called a "silicon-burning quasi-equilibrium", in which these inverse reactions among the alpha-particle nuclei

$$[A = 4N] + ^4\text{He} \rightleftharpoons [A = 4(N+1)] + \gamma, \quad 4N \geq 28$$

as well as many others involving protons and neutrons, do come into equilibrium. Those authors showed that the large abundances in the range $28 \leq A \leq 57$ reflect dramatic-

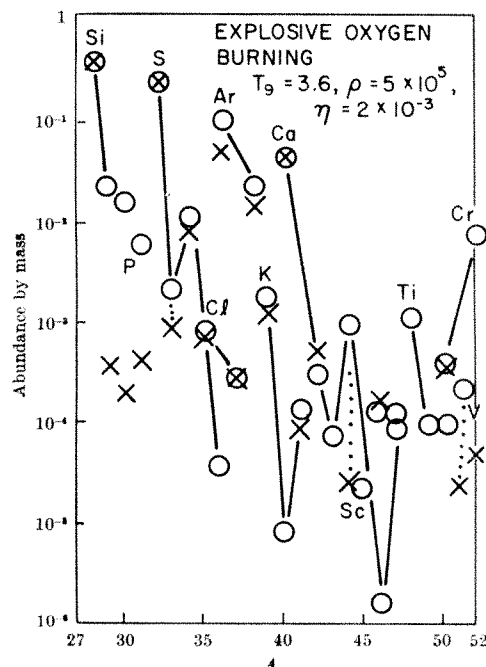


Fig. 3. Products of explosive oxygen burning. The notation is the same as in Fig. 1. The peak temperature is 3.6×10^9 K, the peak density $\rho = 5.0 \times 10^5$ g cm $^{-3}$, and the neutron excess $\eta = 0.002$. Note the excellent reproduction of the ratios of most of the more abundant isotopes.

ally the nuclear properties, basically photoejection thresholds, of the nuclei in that range. Subsequent unpublished calculations by Truran and by Woosley have integrated the reactions of the oxygen burning network through a hotter explosive condition than that shown in Fig. 3, so that the tendency of the expanding matter to freeze into a quasi-equilibrium distribution can be tested. A typical result is shown in Fig. 4, where the final frozen

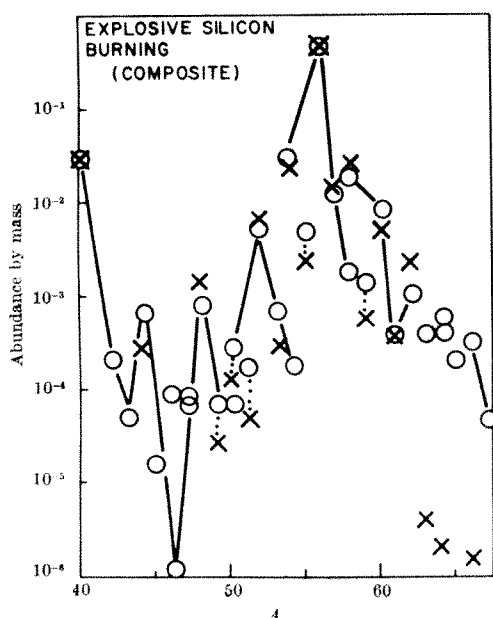


Fig. 4. Products of explosive silicon burning. The notation is the same as in Fig. 1. The calculated abundances shown are a composite of two equal masses of matter processed through extensive, and through complete silicon burning with an explosive history. The peak density in both cases was $\rho = 2 \times 10^7$ g cm $^{-3}$, the peak temperatures were $T = 4.7 \times 10^9$ K and 5.5×10^9 K respectively, and the neutron excess was $\eta = 0.002$ in both cases. The lower temperature case makes ^{56}Ni and the lower- A side of the iron abundance peak while the higher temperature also makes ^{56}Ni as well as the higher- A side of the iron peak.

abundances from oxygen ignition at temperatures near 5×10^9 K are plotted normalized to the solar abundance of ^{56}Fe . The potentially great success in accounting for abundances in the range $44 \leq A \leq 62$ by explosive mass zones similar to this one is self evident.

The overall degree of success in accounting for the nuclear abundances between $A = 20$ and $A = 64$ by explosive nucleosynthesis is summarized in Table 1. Beside each nuclear species is listed its abundance¹¹ on the scale $\text{Si} = 10^6$, the hypothesized explosive source, the abundance calculated by summing the specific results shown in Figs. 1, 3 and 4, and a comment regarding the degree of numerical success we expect to encounter with this approach. The power of ten multiplying each number is enclosed in parentheses. Numerical results for some nuclei are already outstandingly good even though only four specific mass zones of a continuous object have been summed, whereas a few nuclei show only order of magnitude agreement and a few nuclei seem not to be synthesized at all in explosive events. In several cases we have indicated that we anticipate better agreement than was actually obtained by these four mass zones, because our experience so far has shown those particular nuclei to be rather sensitive to changes of conditions. Several important nuclei in this mass range seem not to be due to explosive burning of carbon, oxygen or silicon, and we have indicated that those nuclei are probably due to different circumstances than the ones we have discussed. For example, ^{22}Ne is suspected of being produced during the explosion in the helium burning shells of stars, whereas ^{54}Cr is probably due to the operation of the more quiescent s-process (generally discussed in ref. 12) on ^{52}Cr and ^{53}Cr seed nuclei. Both of these ideas are at present under quantitative investigation. We expect continued rapid progress to inject many subtle new aspects into this outline we are presenting in Table 1.

Astrophysical Comments

The mass zones responsible for the nucleosynthesis we have been discussing have not come from extremely dense objects. If the pre-explosion density were as great as 10^9 g cm $^{-3}$, the temperature would be forced sufficiently high simply to remove the electron degeneracy so that ^{12}C , ^{16}O and ^{28}Si would all be burned into iron-peak nuclei in a time much shorter than the hydrodynamic time scale. To avoid an embarrassing overproduction of Fe relative to those nuclei produced in Figs. 1 and 3 seems to require explosions in massive stars (say, $20\text{--}40 M_{\odot}$), for which the densities are lower and the distribution of mass zones most nearly assumes the required relative proportions. It is not yet clear whether the centre can explode, allowing an outgoing pressure wave to ignite overlying shells, or if the implosion^{7,15} of the evolved core must somehow trigger detonation of non-central shells. The proper dynamic description of the event and the stellar evolution leading up to it remain to be solved quantitatively.

The model of a single event having several concentric mass shells in the differing stages of nuclear burning shown in Figs. 1, 3 and 4 provides an elegant solution to an old problem; why should the relative abundance of nuclei in this mass range remain relatively constant although their absolute concentrations (relative to hydrogen) vary? All that is required is that the relative masses of the explosive carbon-burning, oxygen-burning, silicon-burning and e-process (which is complete silicon burning) shells take on roughly constant proportions in the explo-

Table 1. CALCULATIONS OF EXPLOSIVE NUCLEOSYNTHESIS

Element	A	Solar abundance by mass ¹¹	Explosive process	Calculated abundance	Prospects for agreement
Carbon	12	4.54 (-3)	C-burning	5.0 (-3)	Excellent
Oxygen	16	1.07 (-2)	C-burning	7.01 (-3)	Excellent
Neon	20	1.22 (-3)	C-burning	1.2 (-3)	Excellent
	21	3.62 (-6)	He-burning	?	Uncalculated
	22	1.25 (-4)	He-burning	?	Uncalculated
Sodium	23	4.13 (-5)	C-burning	4.3 (-5)	Excellent
Magnesium	24	5.64 (-4)	C-burning	3.9 (-4)	Excellent
	25	7.54 (-5)	C-burning	9.2 (-5)	Excellent
	26	8.65 (-5)	C-burning	1.0 (-4)	Excellent
Aluminium	27	6.53 (-5)	C-burning	6.9 (-5)	Excellent
Silicon	28	7.34 (-4)	O-burning	8.4 (-4)	Excellent
	29	3.88 (-5)	C-burning	5.2 (-5)	Excellent
	30	2.64 (-5)	C-burning	1.8 (-5)	Excellent
Phosphorus	31	1.12 (-5)	C-burning	3.0 (-6)	Excellent
Sulphur	32	4.37 (-4)	O-burning	4.8 (-4)	Excellent
	33	3.41 (-6)	O-burning	1.6 (-6)	Excellent
	34	2.07 (-5)	O-burning	1.5 (-5)	Excellent
	36	7.06 (-8)	Seed reactions	?	Promising
Chlorine	35	1.48 (-6)	O-burning	1.3 (-6)	Excellent
	37	5.05 (-7)	O-burning	5.0 (-7)	Excellent
Argon	36	1.97 (-4)	O-burning	9.2 (-5)	Excellent
	38	3.90 (-5)	O-burning	2.6 (-5)	Excellent
	40	2.28 (-8)	Seed reactions	?	Promising
Potassium	39	3.35 (-6)	O-burning	2.2 (-6)	Excellent
	40	1.40 (-8)	Seed reactions	?	Promising
Calcium	41	2.60 (-7)	O-burning	1.6 (-7)	Excellent
	40	8.11 (-5)	O-burning	1.6 (-4)	Excellent
	42	5.61 (-7)	O-burning	9.2 (-7)	Excellent
	43	1.31 (-7)	Uncertain	?	Uncertain
	44	1.90 (-6)	Si-burning	8.0 (-7)	Excellent
	46	3.18 (-9)	Seed reactions	?	Promising
	48	1.86 (-7)	Seed reactions	?	Promising
Scandium	45	4.22 (-8)	Uncertain	?	Uncertain
Titanium	46	2.38 (-7)	O-burning	3.0 (-7)	Excellent
	47	2.24 (-7)	Uncertain	?	Uncertain
	48	2.32 (-6)	Si-burning	3.5 (-6)	Excellent
	49	1.77 (-7)	Si-burning	7.8 (-8)	Excellent
	50	1.75 (-7)	Seed reactions	?	Promising
Vanadium	50	1.04 (-9)	Seed reactions	?	Promising
Chromium	51	4.34 (-7)	Si-burning	1.3 (-7)	Excellent
	50	7.61 (-7)	O- and Si-burning	9.6 (-7)	Excellent
	52	1.54 (-5)	Si-burning	1.8 (-5)	Excellent
	53	1.78 (-6)	Si-burning	8.6 (-7)	Excellent
	54	4.56 (-7)	s-process	?	Promising
Manganese	55	1.38 (-5)	Si-burning	6.2 (-6)	Excellent
Iron	54	7.97 (-5)	Si-burning	5.9 (-5)	Excellent
	56	1.30 (-3)	Si-burning	1.3 (-3)	Excellent
	57	3.16 (-5)	Si-burning	4.0 (-5)	Excellent
	58	4.78 (-6)	s-process	?	Promising
Cobalt	59	3.86 (-6)	Si-burning	1.5 (-6)	Excellent
Nickel	58	5.11 (-5)	Si-burning	6.7 (-5)	Excellent
	60	2.05 (-5)	Si-burning	1.6 (-5)	Excellent
	61	9.44 (-7)	Si-burning	1.0 (-6)	Excellent
	62	2.94 (-6)	Si-burning	5.9 (-6)	Excellent
	64	8.99 (-7)	Seed reactions	?	Promising

sions of massive stars responsible for most of the element production. Such a situation may be nothing more than the natural outcome of the physics governing star formation and evolution.

There is an important exception to the constancy of element abundance ratios; the odd Z elements (which are neutron-rich) require initial neutron excess for their production, as was shown in Fig. 2. Inasmuch as the neutron excess probably results primarily from the operation of the CNO cycle, which is itself a second generation feature of any galaxy the chemical evolution of which has proceeded from a starting point of hydrogen and helium, we would expect the earliest galactic nucleosynthesis to have occurred with smaller values of the neutron enrichment parameter η . This effect would lead one to expect the abundances of the elements Na, Al, P, Cl, K, V and Mn to increase with time in the galaxy less rapidly near the beginning than the elements Mg, Si, S, A, Ca, and perhaps Cr and Fe. An observational test for this effect

consists of ascertaining whether old metal deficient stars are more deficient in the former group of elements than in the latter. They are indeed found to be so, the most dramatic case being the recent study of +39°4926 by Kodaira, Greenstein and Oke¹⁶. In Table 2 we show their abundances of the elements Na, Mg, Al and Si on the scale $\log N_H = 12$, with the solar abundances shown for comparison. Whereas Mg and Si are deficient by factors of near thirty in this old star, the neutron-rich elements Na and Al are deficient by factors of several hundred, confirming that they were produced less efficiently in the beginning. Similar though less extreme correlation can be found in the metal deficient stars HD 122563, HD 161817 and HD 140283 (see ref. 16 for discussion and references). It seems likely that a wholly new diagnostic technique for the early chemical evolution of the galaxy is unfolding.

Table 2. ABUNDANCES¹⁶ IN +39°4926 AND IN THE SUN

Element	Log $N_{+39^\circ 4926}$	Log N_{sun}	$\Delta \log N_{+39^\circ 4926}$
Na	3.80	6.30	-2.50
Mg	5.69	7.36	-1.67
Al	3.23	6.20	-2.97
Si	6.02	7.45	-1.43

One of the most exciting aspects of the theory of explosive nucleosynthesis is that it may be subject to a direct experimental check through gamma-ray astronomy. Several of the prominent nuclei, especially ⁵⁶Fe, are synthesized in the form of radioactive parents, in this case ⁵⁶Ni, which are ejected from the explosion before decaying. In the decay a characteristic gamma-ray spectrum is radiated. Its detection both in individual objects¹⁷ and in the integrated background of the universe¹⁸ seems likely, and could place the science of nucleosynthesis on a firmer observational basis.

The past two years have been the most creative ones for the science of nucleosynthesis since the late 1950s. We have attempted to survey these new developments in the theory, to present an original table of the origin of the nuclei in the mass range $20 \leq A \leq 64$, and to emphasize two new diagnostic opportunities for observational astronomy.

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Radio Source Counts from the Ohio Survey

by

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The Ohio survey shows a decrease in the number of faint sources relative to the number which would be expected for a uniform, Euclidean universe. This confirms the results of the Cambridge 5C2 survey at 408 MHz, but the decrease is sharper for the Ohio counts at 1415 MHz.

THREE instalments of the Ohio Sky Survey¹⁻⁴ list about 8,100 radio sources with flux densities above 0.16. (Flux densities (S) are expressed in units of 10^{-26} W m⁻² Hz⁻¹.) These observations were made at a frequency of 1415 MHz and cover an area of about 4.2 steradians, providing the deepest and most extensive survey at frequencies above 408 MHz and the largest number of sources catalogued at any one frequency. These data are used to derive an incremental number-flux density relation which is corrected for effects of noise and confusion. In the usual number-flux density presentation all sources are counted above given flux densities. We have chosen to count sources within given flux density increments, however, so that adjacent number counts and their statistical errors are independent. The index of this incremental relation is 2.5 for a uniform, static, Euclidean universe. For $S_{1415} > 1$ ($S_{408} > 2.4$) the index is 2.7 for the Ohio survey which is in agreement with the Cambridge 4C survey at 178 MHz (refs. 5 and 6), the Cambridge surveys at 408 MHz (5C2 and BP, refs. 7 and 8) and the Bologna (B2) survey at 408 MHz (ref. 9). For $0.16 < S_{1415} < 0.4$ ($0.38 < S_{408} < 0.96$) the index for the Ohio counts decreases to 1.8 or less. This agrees with the trend shown in the 5C2 and B2 surveys at 408 MHz but the decrease in the Ohio count is more dramatic.

The clustering of radio sources in the plane of the sky is investigated. At high galactic latitudes the Ohio sources show no evidence for anisotropy on scales greater than a few degrees but the source density is greater near the North Galactic Spur and within 30° of the galactic plane. Thus any anisotropy in the plane of the sky seems to be associated only with the galaxy and not with extra-galactic objects.

Observations

The Ohio survey was conducted at 1415 MHz using the 260 foot by 70 foot radio telescope¹⁰ of Ohio State University (OSU). The receiver is a Dicke-type radiometer with a refrigerated parametric pre-amplifier¹¹. The resulting system noise temperature is less than 100 K and the r.m.s. fluctuations are 0.025 K consistent with the 8 MHz bandwidth and time constant of 9 s.

The data retrieval and analysis are by automatic digital computer except for manual inspection of the computed output and elimination of spurious sources. The data reduction is based on procedures developed primarily by Dixon¹². Many of the details are given with the published source lists. Essentially uniform observation and reduction procedures were used throughout the survey. Fig. 1 shows the sources found in the regions of sky covered in the three instalments of the Ohio survey used in the present study. Some numerical data for these instalments are given in Table 1. Sky areas surrounding some very strong sources (including the Sun) were omitted from the survey. The flux density scale adopted is within 5 per cent of that adopted in the NRAO catalogue¹³ or essentially the same as that defined by Kellermann¹⁴.

Corrections

There are two principal effects which cause systematic differences between the observed and true number counts. These are:

(a) Errors in measured flux density due to both system noise and errors in the reduction procedure. Comparison of the flux densities of Ohio sources having small angular diameters and $S > 1$ with the accurate values given in the

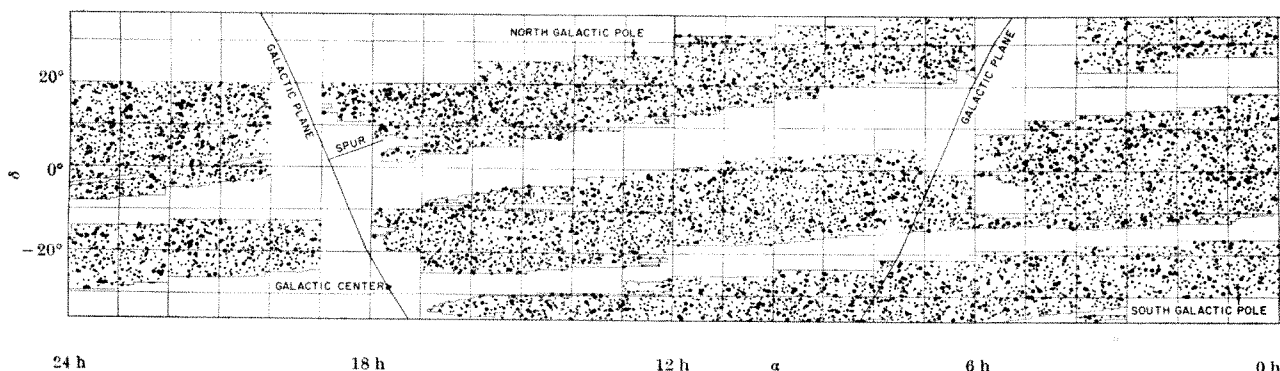


Fig. 1. Radio sources in the regions of sky covered in three instalments of the Ohio survey between +37° and -36° declination (δ) and 0 to 24 h right ascension (α) (epoch 1950.0) at 1415 MHz. The large dots represent sources of $S_{1415} > 1$ (flux unit), the medium size dots represent sources of $0.5 < S_{1415} < 1.0$ and the small dots represent sources of $S_{1415} < 0.5$. Some clustering of sources near the (North Galactic) Spur and the galactic plane is apparent. The presentation is on a rectangular projection for which the density will show a $\cos \delta$ effect of 25 per cent maximum for the regions covered. This $\cos \delta$ effect was accounted for in the clustering studies discussed in the text.

Table 1. DATA FOR THREE INSTALLMENTS OF THE OHIO SURVEY

Instalment	Dec. range	R.A. range	Area (Sr)	$S_{1415} > 0.16 \times 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$ Total No. of sources	No. of sources per steradian	$S_{1415} > 0.2 \times 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$ Total No. of sources	No. of sources per steradian
II	+19° to +37°	00 h to 16 h	0.64	1,200	1,800	988	1,540
III	00° to 20°	00 h to 24 h	1.22	2,325	1,910	2,070	1,700
IV	00° to -36°	00 h to 24 h	2.36	4,550	1,930	3,777	1,600
Total			4.22	8,075	1,915	6,835	1,620

References: instalment II: Dixon and Kraus¹; instalment III: Fitch, Dixon and Kraus², Fitch³; instalment IV: Ehman, Dixon and Kraus⁴. The first instalment of the survey by Scheer and Kraus⁵ is not included here as it was restricted to sources with $S_{1415} > 0.37 \times 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$.

NRAO catalogue, by Fomalont¹⁵ and by Harris¹⁶, gives an r.m.s. proportional error in flux density of about ± 7 per cent. For the weaker sources confusion errors predominate.

(b) Confusion resulting from the finite resolution of the radio telescope. Included in this category are the following effects: (1) Flux density errors due to many sources below the flux density limit of the survey. These may be roughly represented by a random distribution of standard deviation 0.05. This value was found from an examination of the survey records, and is in agreement with that calculated from an extrapolation of the number-flux density relation to low flux densities. (2) Errors due to confusion by sources near and above the lower flux density limit of the survey, depending partly on the reduction procedure. (3) Masking of the weaker sources by stronger ones. (4) Small scale angular clustering. The effect of source clustering on the number-flux density relation has been shown by Pooley and Ryle¹⁷ to be unimportant on scales greater than several minutes of arc. Most apparent doubles can be explained by a random distribution of radio sources. Physical doubles with separation less than several minutes of arc will be counted as single sources in the beam area of the OSU telescope and have no significant effect on the source counts.

The net corrections to the number counts may be best determined by a complete Monte Carlo analysis (ref. 18 for example). We have taken a simpler approach and deduced the corrections due to masking (part b3) from the partial Monte Carlo analysis by Dixon¹². The effects of the combined errors in flux density due to noise and confusion (part a and parts b1 and b2) have been calculated by convolving the combined flux density error distribution with the number-flux density relation. The latter procedure is similar to that considered by Bennett¹⁹. The results of these calculations are summarized in Table 2, columns 2 and 3 respectively. Column 4 gives the total correction to the number counts determined from the values in columns 2 and 3. The estimated r.m.s. uncertainties are also given in the table.

In deriving a number-flux density curve of cosmological significance, it is necessary to exclude galactic sources. Our investigation of the distribution of sources as a function of galactic latitude indicates that possibly 7 per cent of the total number of the Ohio sources may be galactic. The exact form of their number-flux density relation is unknown. Their inclusion in the number counts will not influence the final result significantly, however.

Number-Flux Density Relation

The observed number-flux density counts for the Ohio survey are given in Table 3. The Ohio number counts per unit flux density per steradian relative to those expected in a universe model for which $n_0 (S_{1415}) = 376 S_{1415}^{-2.5}$ are presented in Fig. 2 by the heavy solid curve with flux densities at 1415 MHz indicated by the inserted abscissa scale. Arrows show by their length the magnitude of the correction. At high flux densities statistical errors ($\propto n(S)^{-1/2}$) predominate. At low flux densities the errors are due to the uncertainties of the systematic corrections. Individual graphs for the three instalments of the survey show no significant differences. The index of the number-flux density relation is 2.7 for $S_{1415} > 1$ decreasing to 1.8 or less for $S_{1415} < 0.4$. These values should be compared

with an index of 2.5 for counts in a uniform, static, Euclidean universe.

Comparison with Other Surveys

We have taken count data from the Cambridge 4C survey at 178 MHz and the Cambridge (5C2 and BP) and Bologna (B2) surveys at 408 MHz. In Table 4 the observed counts for these surveys and the Ohio survey at 1415 MHz are listed for 20 per cent flux density increments. The 178 and 1415 MHz counts were transformed to 408 MHz assuming a Gaussian spectral index distribution having a mean value of 0.7 so that all five surveys can be inter-compared at 408 MHz as shown in Fig. 2. All counts are relative to the same uniform, static Euclidean universe model for which the number per unit flux density per steradian is given by $n_0 (S_{408} = 1385 S_{408}^{-2.5})$. The arrows show by their length the correction for noise and confusion and where possible are those estimated by Gower¹⁸ and Pooley and Ryle¹⁷. For $S_{408} > 1$ the number-flux density curves are in substantial agreement. For $S_{408} < 1$ the Ohio count is significantly below those of the Cambridge 5C2 and Bologna B2 surveys.

Clustering

We have tested the significance of differences between the observed distribution of Ohio sources over the sky and a random distribution. The area covered by the Ohio survey has been divided into boxes of three sizes: 5°, 10° and 20° square. The numbers of sources in each box have been counted for two ranges of flux density $0.2 < S_{1415} < 0.4$ and $S_{1415} > 0.4$. The numbers of boxes containing n sources have been compared with those expected on a Poissonian distribution. Statistical chi-squared tests show that no significant clustering occurs on angular

Table 2. CORRECTION OF OBSERVED NUMBER COUNTS TO OBTAIN TRUE NUMBER

Flux density at 1415 MHz $\times 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$	Correction due to masking (per cent)	Correction due to flux density errors (per cent)	Total correction (per cent)
0.89	+9 ± 4	-20 ± 10	-13 ± 11
0.36	+22 ± 6	-37 ± 30	-23 ± 31
0.13	+65 ± 8	-31 ± 25	+12 ± 26

Table 3. OBSERVED NUMBER-FLUX DENSITY RELATION FOR THE OHIO SOURCES

Lower limit for 20% flux density increment†	Observed counts	Observed counts per steradian	Observed counts relative to model*
0.162	914	216	0.27
0.194	1,098	261	0.42
0.233	1,022	242	0.52
0.279	928	220	0.62
0.335	756	179	0.66
0.402	754	179	0.87
0.482	605	143	0.91
0.579	447	106	0.91
0.696	408	96.6	1.07
0.833	305	72.2	1.05
1.00	208	49.3	0.94
1.20	178	42.1	1.07
1.44	115	27.2	0.91
1.73	92	21.8	0.95
2.07	54	12.8	0.74
2.49	54	12.8	0.96
2.99	34	8.1	0.80
3.58	19	4.5	0.59
4.30	21	5.0	0.85
5.16	16	3.8	0.86
(> 6.19)	(25)	(5.9)	

* Euclidean model counts per unit flux density per steradian are given by $n_0(S_{1415}) = 376 S_{1415}^{-2.5}$.
† In units of $10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$ at 1415 MHz.

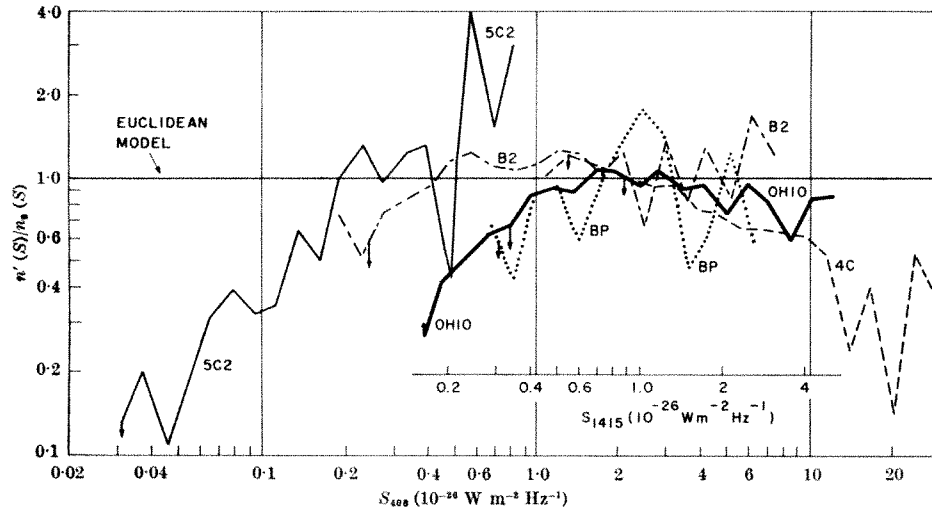


Fig. 2. Observed number counts of the Ohio and other surveys compared at 408 MHz relative to the number expected for a uniform, static Euclidean universe (given by the horizontal line for $n'(S)/n_e(S) = 1$ for our model) as a function of the flux density (lower limit of 20 per cent increment). At low flux densities the Ohio counts decrease below the Euclidean model more rapidly than the Cambridge 5C2 and Bologna B2 counts. The Ohio sources were observed at 1415 MHz (flux density scale insert) and transformed to 408 MHz (main scale) assuming a Gaussian spectral index distribution having a mean value of 0.7. The five surveys are the Ohio, the Cambridge 4C, 5C2 and BP, and the Bologna B2.

scales of 5° , 10° or 20° except in the region of the North Galactic Spur.

This method does not provide a very sensitive test of clustering so we examined the number distribution of the boxes on the plane of the sky. The 5° boxes containing ten or more strong or weak sources do cluster and the regions are found to contain many extended sources (Ehman, private communication). Their concentration increases toward the galactic plane and the North Galactic Spur, resulting in an excess of about 15 per cent in the number counts within 30° galactic latitude. Their detailed distribution is under investigation. The number-flux density relation of these sources is not well known. Their inclusion in the source statistics will have only a small effect, however.

Further attempts to correlate areas of high source density with Abell clusters and high velocity hydrogen clouds were made, with negative results.

Conclusions

The Ohio counts involving 8,100 sources confirm the trend reported by Ryle²⁰ for the Cambridge surveys

involving a smaller number of sources. For $S_{408} > 1$ ($S_{408} > 2.4$) the number-flux density index is 2.7 while for $S_{408} < 0.4$ ($S_{408} < 0.96$) the index for the Ohio and Cambridge surveys decreases to 1.8 or less. In the Ohio survey (at 1415 MHz) there is a sharper decrease in index at low flux densities, however.

Numerical calculations by Longair²¹ for an Einstein-de Sitter universe show that such a cut-off in numbers of fainter sources may be explained by a sharp decrease in source densities at redshifts $z \approx 4$. Longair finds that the steep slope of the number-flux density relation for later epochs is explained by incorporating either luminosity or density evolution for the most luminous sources.

There is general agreement between the Bologna B2 survey at 408 MHz and the Cambridge surveys at this frequency. This has permitted the difference in source counts with respect to the Ohio survey at low flux densities to be investigated by comparing sources common to both the Ohio and Bologna B2 surveys. Spectral indices between 408 and 1415 MHz have been calculated for 200 unconfused sources having $0.4 < S_{408} < 1.0$. Their distribution is shown in Fig. 3a. Spectral indices between 408 and

Table 4. OBSERVED NUMBER-FLUX DENSITY COUNTS FOR THE OHIO SURVEY, THE CAMBRIDGE 4C, 5C2 AND BP SURVEYS, AND THE BOLOGNA B2 SURVEY

Survey	Ohio	4C	5C2	BP	B2
Frequency (MHz)	1415	178	408	408	408
Lowest flux density ($\times 10^{-26}$ W m ⁻² Hz ⁻¹)	0.16	2.0	0.02	0.70	0.20
Area (steradian)	4.2	6.9	0.0039	0.15	0.44
20% increment	Number	Number	Number	Number	Number
1	914 (0.162)*	$\approx 1,195$ (1.94)	11 (0.022)	34 (0.695)	≈ 725 (0.194)
2	1,098 (0.194)	1,085 (2.33)	11 (0.026)	17 (0.833)	384 (0.233)
3	1,022 (0.233)	793 (2.79)	19 (0.031)	25 (1.00)	428 (0.279)
4	928 (0.279)	588 (3.35)	21 (0.037)	21 (1.20)	359 (0.335)
5	756 (0.335)	393 (4.02)	9 (0.045)	10 (1.44)	304 (0.402)
6	754 (0.402)	282 (4.82)	12 (0.054)	13 (1.78)	289 (0.482)
7	605 (0.482)	218 (5.79)	15 (0.065)	13 (2.07)	239 (0.579)
8	447 (0.579)	134 (6.95)	14 (0.078)	14 (2.49)	158 (0.695)
9	408 (0.695)	99 (8.33)	9 (0.094)	8 (2.99)	117 (0.833)
10	305 (0.833)	66 (10.0)	7 (0.112)	2 (3.58)	94 (1.00)
11	208 (1.00)	50 (12.0)	10 (0.135)	2 (4.30)	81 (1.20)
12	178 (1.20)	37 (14.4)	6 (0.162)	3 (5.16)	60 (1.44)
13	115 (1.44)	27 (17.3)	9 (0.194)	1 (6.20)	38 (1.73)
14	92 (1.73)	18 (20.7)	9 (0.233)	0 (7.43)	35 (2.07)
15	54 (2.07)	6 (24.9)	5 (0.279)	0 (8.90)	14 (2.49)
16	54 (2.49)	8 (29.7)	5 (0.335)	2 (10.7)	22 (2.99)
17	34 (2.99)	2 (35.8)	4 (0.402)	0 (12.8)	10 (3.58)
18	19 (3.58)	6 (43.0)	1 (0.482)	1 (15.4)	12 (4.03)
19	21 (4.30)	3 (51.6)	7 (0.578)	0 (18.4)	6 (5.16)
20	16 (5.16)	3 (61.9)	2 (0.695)	0 (22.1)	9 (6.19)

*The values in parentheses indicate the lower flux densities of each 20 per cent increment in units of 10^{-26} W m⁻² Hz⁻¹.

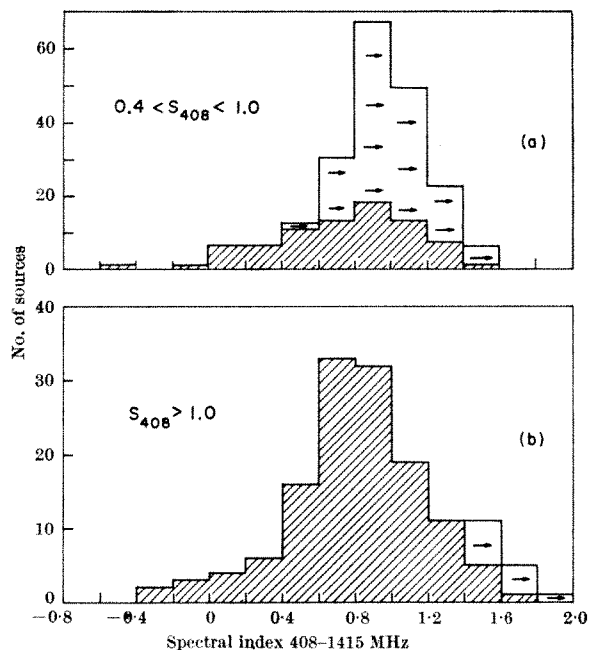


Fig. 3. *a*, Spectral index distribution for 200 Bologna survey sources having $0.4 < S_{408} < 1.0$ for which Ohio 1415 MHz flux densities were available (shaded) or for which no corresponding sources were found in the Ohio survey yielding upper limits for the spectral indices (open). *b*, Spectral index distribution for 143 Bologna survey sources having $S_{408} > 1.0$ for which Ohio 1415 MHz flux densities were available (shaded) or for which no corresponding sources were found in the Ohio survey yielding upper limits (open). A lower limit of $S_{1415} = 0.16$ was taken and at this flux density 60 per cent of the sources should have been found in the Ohio survey.

1415 MHz have also been calculated for 143 sources having $S_{408} > 1.0$. Their distribution is shown in Fig. 3*b*. The open (unshaded) portions of the histograms indicate indices for which only an upper limit could be given, for there were no corresponding sources on the 1415 MHz records. The histogram on Fig. 3*a* for the fainter sources

is displaced toward larger spectral indices as compared with Fig. 3*b* for the stronger sources. This difference could be interpreted as indicating steeper spectra for the fainter sources. This could imply that the more distant and more luminous objects have steeper spectra. Independent evidence has already been advanced that more luminous sources have steeper spectra^{16,22}.

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Pulsar Sub-pulses and the Emission Process

by

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This article describes another regular phenomenon in the sub-pulse behaviour of pulsars. The drift speed of successive sub-pulses follows a sawtooth pattern with a linearly increasing drift speed and sudden jumps as the speed drops to repeat the cycle.

THERE is a great variation of structure from one pulsar pulse to the next, although the mean pulse shape obtained by superimposing a train of pulses with the basic pulsar period P_1 remains constant. In certain pulsars (and perhaps all) these variations can be attributed largely to sub-pulses separated in time by the "second periodicity" P_2 (ref. 1) and drifting past the "windows" of the mean pulse shapes at a rate measured by a "third periodicity", defined as P_3 , equal to the mean time between consecutive appearances of sub-pulses at a given point of the mean pulse window. Because the number of sub-pulses appearing in the windows of a train of pulses varies regularly, the third periodicity also manifests itself² as a peak in the power spectrum of the pulse energies. The fact that a similar sub-pulse structure (F. D. Drake, URSI Assembly,

Ottawa, 1969, and ref. 4) and intensity reappear in several successive pulses suggests that an underlying pattern in the mechanism responsible for sub-pulses persists for this interval. This is borne out by the constancy of the drift rate of any particular chain of sub-pulses, although other chains may have different rates.

The pulsar CP 0808, which has particularly well-defined sub-pulses, has been observed at 81.5 MHz with the four-acre antenna at the Mullard Radio Astronomy Observatory. This is a transit instrument capable of observing sources only while they are within about $\frac{1}{2}^\circ$ of the meridian.

A total power receiver with a bandwidth of 30 kHz was used and the time averaging due to dispersion was 2.8 ms. The period of CP 0808 is ≈ 1.292 s and the mean

pulse length is ≈ 80 ms. The pulses were recorded digitally at 250 samples per second, and Fig. 1 shows some of the observations with each horizontal line representing a time window centred on the mean pulse and with successive windows exactly one pulse period apart. The signal amplitude at succeeding samples 4 ms apart has been quantized so that a circle and cross represent an amplitude greater than twice and three times the standard

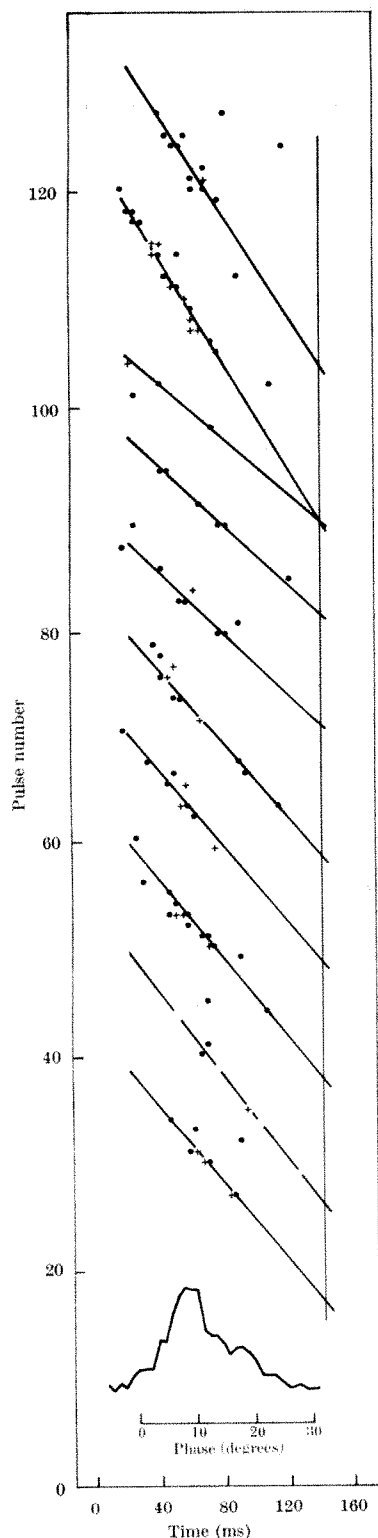


Fig. 1. Sections of successive pulses of CP 0808 sampled every 4 ms and quantized such that O and X represent amplitudes greater than two and three standard deviations of the received noise.

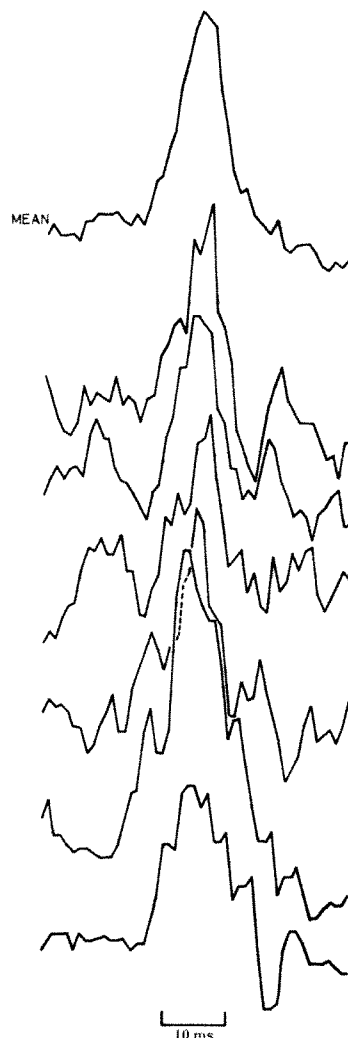


Fig. 2. Six representative mean sub-pulse shapes for individual chains of pulses and the mean of these six aligned by eye.

deviation of the noise on the digitized record. The drifting sub-pulses are clearly seen, separated on average by about 55 ms and taking, on average, 11 pulse periods to drift through this sub-pulse separation. In this, as in all other pulsars showing this phenomenon^{3,4}, the drift is from the trailing edge of the pulse to the leading edge. The plot shows that each drifting sub-pulse chain has its own (constant) drift speed which can differ markedly from the drift speeds of adjacent chains. The correlation of intensities of sub-pulses for any particular chain can also be seen.

The mean pulse shape (Fig. 1) is found by superimposing pulses sampled at intervals of P_1 . The mean sub-pulse shape can be obtained by superimposing successive pulses with shifts appropriate to the drift rate of the sub-pulse chain. The six averages shown in Fig. 2 were aligned by eye and a further average taken to give a more general mean sub-pulse shape for the pulsar. This is almost Gaussian and of half power width ≈ 8 ms. Because the effective time constant of the recording system was approximately 6 ms, the true mean sub-pulse is only 4 or 5 ms in half power width, much less than was suspected from previous studies^{3,4}. The narrowness of the individual average sub-pulses in Fig. 2 implies that the sub-pulse chains deviate by less than 5 ms from a straight line during the time (15 periods or 20 s) for the sub-pulse to cross the mean pulse window. The individual drift rates must then be constant to within 5 ms in 20 seconds or 1 part in 4,000. The drift rates of

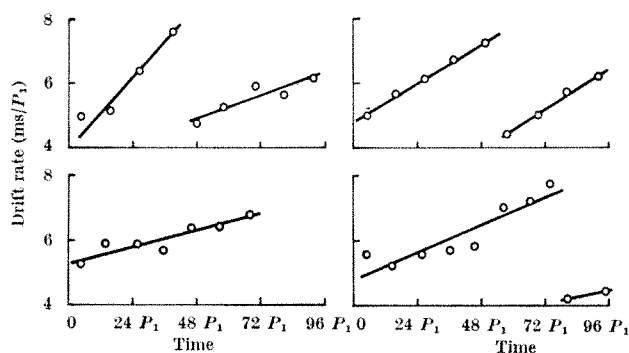


Fig. 3. Plots of the drift rates of successive sub-pulse chains for four two-minute recordings.

different chains, however, vary within the range 4.0 ms per pulse to 7.8 ms per pulse.

The drift rates of successive sub-pulse chains are plotted in Fig. 3 for four recordings of CP 0808 each lasting two minutes, and it can be seen that the drift rates show a sawtooth type of variation with a linear increase of rate followed by a sudden jump to restart the cycle. This behaviour is also revealed if one tries to fit a constant period to the sub-pulse chains at some reference point in the mean pulse window. The residual phase would have a linearly decreasing slope and a jump of about one cycle to repeat the sequence. The phase plots of refs. 1 and 3 show that just such behaviour has already been seen, but not recognized, in CP 0808, AP 2015 and possibly CP 1919. The same behaviour is seen in P_2 which increases up to 80 ms and then jumps as low as 48 ms. The mean value of P_2 is dependent on which part of the mean pulse is used as reference and can be as high as 62 ms at the trailing edge of the pulse. In ref. 3 it was found that for CP 0808 the jump occurs about every 200 pulse periods. It seems that P_3 must be defined with care. If it is defined by observations between jumps, it will be smaller than if it is taken as the average over a long record of data which includes several jumps. The value of P_3 has been found to be $11.1 P_1$ when measured by the sub-pulse drift rate⁴ and to be $11.7 P_1$ when measured by the power spectrum of the intensities of a long succession of pulses². If these two values correspond to the two ways of defining P_3 , there must be a jump every $11.1 \times 11.7 \times P_1 / (11.7 - 11.1) = 220 P_1$, in good agreement with the value given in ref. 3. It seems reasonable then to define a periodicity P_5 as the mean time between jumps in the observed drift rate.

For pulsars MP 0031 and CP 1919 a fourth periodicity P_4 has been observed⁹ which is close to the time for the sub-pulse to drift through the basic pulse period P_1 . It seems that⁹ after the time $P_4 = P_1 P_3 / P_2$, the same sub-pulse could reappear in the mean pulse window. In using the formula for P_4 , the various periods will have to be very carefully defined because they obviously vary throughout the sawtooth cycle.

A theory of pulsars which explains many of the radio observations is that proposed by Radhakrishnan and Cooke⁵ and extended by Komesaroff⁶. In this theory radio emission comes from the region just above the magnetic poles of a rotating neutron star with an inclined magnetic axis. We may suppose that the sub-pulses originate in "concentrations" within this emitting region—an interpretation reinforced by the observations that the polarization of sub-pulses of CP 0328 (ref. 7) and PSR 0833 (ref. 8) is, in general, that of the corresponding section of the mean pulse. A sub-pulse is then seen as the rotating neutron star sweeps the concentration past the observer. For CP 0808 at 81.5 MHz the sub-pulse width of 5 ms and the pulse period of 1.292 s imply that the angular width of the beam from the concentration is only 1.5° . Any theory of the generation of the radio

pulses must provide this form of pulse and not that of the mean pulse shape. The narrow beam and constant drift rate also require that the (hour angle) directions of this beam vary by no more than a fraction of a degree from some fixed angular relation to the pulsar meridian.

The problem is then to explain (i) the presence of a region producing a radio beamwidth of only 1.5° , (ii) the maintenance of this region for at least 20 s, (iii) the movement of the region through the pulse window with a speed constant to better than 1 part in 4,000, and (iv) the sawtooth behaviour of the sub-pulse drift rate.

The marching sub-pulse behaviour has been ascribed⁹ to (density) fluctuations distributed quasi-periodically around the circumference of a revolving circumpolar plasma and the corotation radius was suggested as the most plausible site for these. But the strict requirement on the beam direction of the sub-pulse and the direct relation between sub-pulse polarization and mean polarization argue rather for a system rigidly tied to the surface of the neutron star.

Consider then a rotating neutron star with inclined magnetic axis and emission restricted to within a cone around the magnetic poles. The line of sight to the pulsar sees a section through a cone once every rotation of the neutron star—every P_1 , that is. The actual section of the cone scanned by the observer defines the mean pulse shape while the magnetic field directions along the scan define the mean polarization properties of the pulsar⁶.

The presence of successive sub-pulses separated by an angle represented by P_2 and drifting through the mean pulse window every P_3 with increasing speeds is then compatible with the concentrations responsible for the sub-pulses being in a layer undergoing differential rotation and having successive concentrations enter the

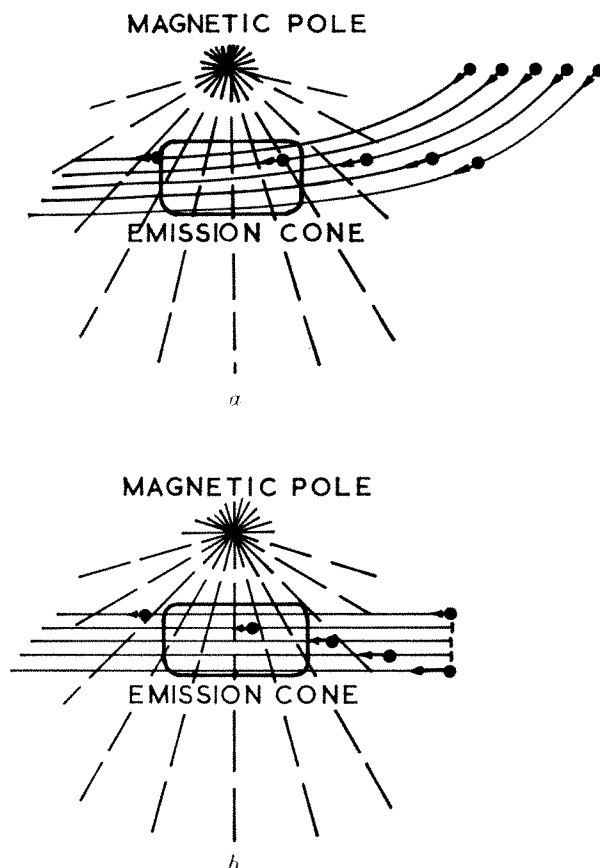


Fig. 4. The proposed explanation of the sub-pulse behaviour and an illustration of how the magnetic field latitude is a function of pulsar latitude.

cone at lower and lower pulsar latitudes. There may be a parallel with the motion of sunspots on the Sun which have a rotational period which varies with solar latitude and which appear at lower solar latitudes during the solar cycle. In physical terms the concentrations could be regions injecting plasma out along the magnetic field lines which cut the region.

One can envisage two possibilities for the drift of the concentrations through the cone. They may either descend in latitude or drift at the same latitude. In principle these alternatives could be distinguished by the curvature of the resulting sub-pulse chains in a plot such as that of Fig. 1. In practice, however, the expected differences of curvature are far below the noise level. During its lifetime, the sub-pulse concentration which is assumed to circulate the neutron star to produce P_4 could be at a lower latitude, and hence drift at a faster speed, on its second appearance. This should be capable of observational verification.

Projection of the sub-pulse chains back in time would be expected to give some clue about their generation. If the concentrations descend in latitude as they drift in longitude they may originate along a line at constant latitude as sketched in Fig. 4a. It is difficult in this case, however, to account for the regularity of the jumps in drift rate.

Whether or not the concentrations drift in latitude, they could be projected back to a region of constant longitude at which the concentrations are created at progressively lower latitudes, another cycle starting again at higher latitudes. It can be seen from Fig. 1 that, at a time equivalent to a longitude 30° before the centre of the mean pulse window, the chains of sub-pulses would be repeating at regular intervals (P_3). For CP 0808 $P_3 \approx 14$ s and it may be significant that, for a typical neutron star, this time is of the order of that for an Alfvén wave to travel along a diameter.

Other theories of pulsars have been proposed by Eastlund¹⁰ and Gold¹¹ in which particles trapped in the magnetic field lines of the magnetosphere emit synchrotron radiation seen as pulses as the neutron star rotates. For particles trapped in the Earth's magnetosphere there is an east to west movement of the particle due to the geometry of the field¹². Such a movement

could produce the drift of the sub-pulse, but the sawtooth behaviour is not an obvious feature of these theories either.

The structure of the magnetic field within the emission cone determines the polarization properties of the emitted pulse. The structure must be a function of latitude (Fig. 4) so that it is predicted that the polarization properties of pulses would depend on the relative phase of P_5 . Indeed, it should be possible to trace the path of the sub-pulse through the window by the measurement of polarization angle of successive sub-pulses.

Many pulsars have not yet been observed to have second periodicities. If the cone of emission included many concentrations or they were of long enough lifetime to circulate many times around the pulsar, the resulting sub-pulse behaviour would be greatly confused. Similarly, if the cone's latitudinal extent was large, the polarization properties would vary greatly throughout the cycle of any P_5 and the strong polarization which might be apparent on individual pulses would be smeared in the mean.

The periodicity structure is the clue to the atmosphere of the pulsar, and the sub-pulse shape is the chief clue to the radio emission mechanism. What is needed is a knowledge of the sub-pulse shape and its behaviour as a function of frequency and from pulsar to pulsar. For example, the ratio of sub-pulse width to pulse period is about 0.006 for at least CP 0808, AP 2015 and CP 0328.

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Superfluid Turbulence in Neutron Stars

by

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The neutron superfluid in most neutron stars should be in a highly turbulent state. If so, this turbulence drastically alters its rotational properties.

A CLASSICAL viscous liquid placed in a uniformly rotating container will (in the steady state) rotate uniformly with the container. Superfluid helium in the same container will rotate as follows^{1,2}. Vortex lines are formed, uniformly distributed through the liquid and all parallel to the rotation axis. They rotate with the container as if they formed a rigid array. The helium flows about the lines in vortex motion. If an average is taken of the superfluid velocity field over many vortex lines the motion appears classical, that is, there is rigid body rotation. Depending on the roughness of the container surface the vortex lines either do or do not firmly attach themselves to it.

Now imagine that the container is steadily slowing down, and that its surface is such that the vortex lines are firmly pinned to it. Because the superfluid is inviscid it will rotate faster than the container. The vortex lines move with the superfluid, but are pinned to the walls; initially straight, they are therefore steadily lengthening and wrapping about the container. The situation is shown schematically in Fig. 1. Every time the superfluid laps the container by 2π radians, the vortex lines wrap once more about the axis. Eventually the wrapping is very tight. Along the equator lines of opposite sense are brought near each other. This situation is unstable; eventually two opposing lines will be brought sufficiently close to

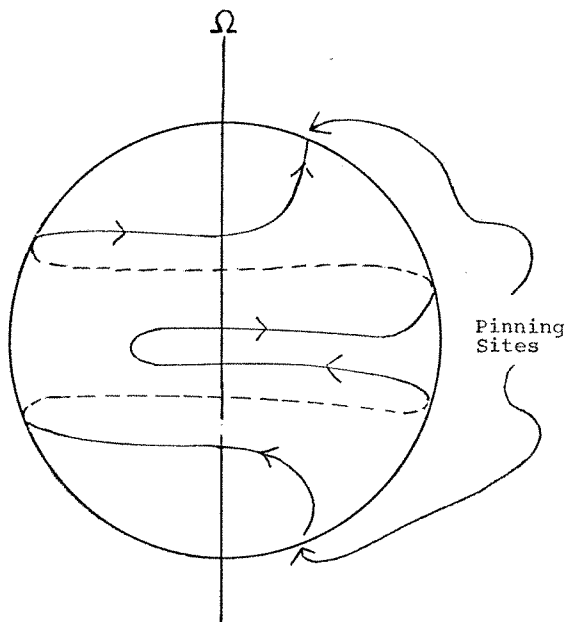


Fig. 1. A rotating superfluid in a less rapidly rotating spherical container. One vortex line is shown. (The arrow on the line represents the sense of vortex motion of the superfluid about the line.) Initially parallel to the rotation axis, it is wrapped as shown after the fluid has lapped the container by 2π radians. The line length has increased in this process, but the projection of this length onto the rotation axis has not.

reconnect, forming a vortex ring and a shorter vortex line (Fig. 2). The ring migrates away, the line steadily lengthens, new rings form and the process continues. As a given ring migrates about, it collides with others and with lines, exchanging impulse with them. Waves will be excited in the vortex lines and rings. Eventually a ring will be broken³ into two smaller rings. These rings will migrate faster, collide more often, and break sooner into still smaller rings. Feynman³ speculates that the ring of smallest possible diameter is in fact a roton. If so, the breaking up of large rings leads in the end to the heating of the fluid. The whole process constitutes a superfluid version of the dissipation of velocity differentials into heat. In the steady state the superfluid contains tightly wrapped vortex lines, no longer parallel to the rotation axis, plus large numbers of vortex rings. This state is the superfluid version of fully developed turbulence.

It is possible that the neutrons within a neutron star form a superfluid (refs. 4–6, unpublished work of Ruderman, and the refs. contained therein). The region of neutron superfluidity begins within or just below the crystal lattice (unpublished work of Ruderman, refs. 5, 7, 8, and the refs. contained therein) thought to form the outer layers of neutron stars and extends up to densities of $\approx 3 \times 10^{14} \text{ g cm}^{-3}$. In the cores of the more massive neutron star models higher densities are reached, and hyperons are present in great numbers. The question of neutron (and hyperon) superfluidity in these cores is unresolved. The protons, but not the electrons, may form a superconductor.

Vortex lines in liquid helium pin effectively to container walls if the walls possess irregularities of the same general size as the vortex cores. The "wall" in the case of a neutron star to which the neutron vortex lines might pin is the crystal, largely composed of nuclei with Z in the range 30–50. The nuclear sizes are then $\approx 5 \times 10^{-13} \text{ cm}$, roughly comparable⁴ with the radii of the vortex cores ($\approx 10^{-12} \text{ cm}$). The thermal vibrational motions of the nuclei in the crystal are small (it is far below its melting temperature). The neutron vortex lines therefore might be expected to pin to the lattice. The lattice, of course,

is slowing down; it is firmly tied to the magnetic field, which constitutes the "pulsar", and pulsars are slowing down. The neutron superfluid within a neutron star might therefore be in a highly turbulent state. If so, this turbulence would have a variety of effects.

(Superfluid turbulence should also occur even if vortex lines do not pin effectively to the crust. This is because the charged particles within a neutron star, which co-rotate with the crust, exert a non-uniform force on each line. This force arises from the scattering of the charges against the neutrons in each line's normal core. The radius of this core, the neutron coherence length, varies with depth within the star, so does the cross-section for scattering, and consequently the force per unit length on the line. The gradient of this force constitutes the shearing force acting steadily to lengthen the line.

(Baym *et al.*¹⁶ have evaluated the force per unit length f on a vortex line due to the scattering of electrons off the neutron magnetic moments. Their result (equation (9) of ref. 16: f is inversely proportional to the relaxation time they give) can be cast in the form

$$f \propto \{n^{1/3} \Delta / \epsilon\}^{-2}$$

where n is the number density of neutrons, ϵ their Fermi energy and Δ the neutron superfluid energy gap. Δ is given in ref. 6. It varies with position by a factor of ≈ 10 . The value of f then calculated varies with position by a factor of $\approx 10^3$. This strong variation ensures that superfluid turbulence will occur.)

(i) Ruderman⁹ has proposed that the long-period "wobble"¹⁰ in the period of the Crab pulsar might be due to a slow oscillation of the neutron vortex lines. The lines are taken to be parallel to the rotation axis and free to move axially. Clearly, if they are pinned to the crust and tightly wrapped, such a mode of oscillation is strongly modified; very likely it is impossible.

(ii) It is well known that fully developed turbulence possesses a turbulent kinematic viscosity $\nu = (\text{mean velocity in a typical eddy}) \times (\text{diameter of the typical eddy})$. But the velocity field of a superfluid about a vortex line is $v = n\hbar/mr$ with $n = 1, 2, 3, \dots$. Therefore $\nu \approx n\hbar/m$. Here m is twice the neutron mass and \hbar is Planck's constant. (This estimate is in agreement¹¹ with observed properties of the turbulent flow of superfluid helium.) This viscosity dissipates any velocity differentials that might exist in the fluid over distances D in a time $t \approx D^2/\nu \approx 500 D^2/n \text{ s}$

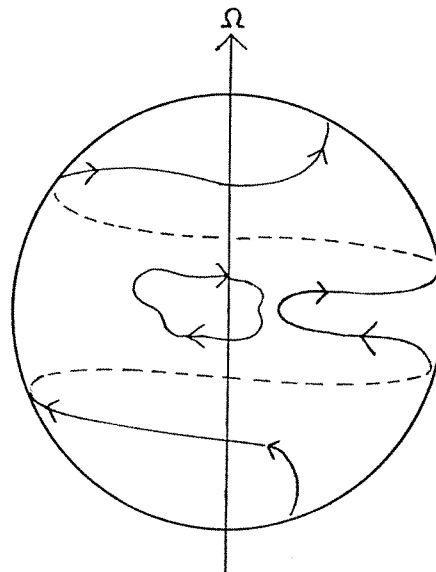


Fig. 2. The line in Fig. 1 has reconnected at the equator, shortening the line and forming a vortex ring.

(D in cm). In particular, a region of thickness $\approx \sqrt{n\Omega/500\Omega} (\approx 1/100 \text{ km for the Crab pulsar})$ will be formed just below the outer crystal in which the neutron superfluid corotates with the crystal. The moment of inertia of this layer can be comparable with that of the crystal. (In the event of a starquake^{12,13}, of course, only a very thin layer of superfluid is suddenly accelerated.)

The existence of this boundary layer, produced by turbulent viscosity, fulfils the conditions required for Eckman pumping to begin. This process spins down the neutron superfluid in a time¹⁴ $t_E \approx R/\sqrt{\nu\Omega} \approx 1/\sqrt{n\Omega} \text{ yr}$, where R is the stellar radius. This time scale (one month for the Crab pulsar, perhaps one year for the slowest pulsars) is always short compared with that for the slowing down of the crust. The neutron superfluid, then, will almost exactly corotate with the crust, maintaining only a small velocity differential with it in order to maintain its turbulent state. (Were the velocity differential to go to zero the vortex lines would no longer be continuously stretching; the turbulence would then dissipate away and Eckman pumping would cease.)

(iii) Vortex lines are in a state of tension, the tensional force being $F = \pi \rho (\hbar/m)^2 \ln(d/\xi)$ where $\rho \approx 10^{14} \text{ g cm}^{-3}$ is the superfluid density, d the distance between vortex lines and $\xi \approx 10^{-12} \text{ cm}$ the vortex core radius. The total number of neutron vortex lines in a neutron star is $N \approx 4 \times 10^{15} \Omega$, where Ω is the angular velocity of the neutron superfluid (nearly equal to that of the pulsar). The total torque exerted by the lines on the crust, and through it to the pulsar, is NFR . The moment of inertia of the crust-plus-charged particles is typically $\approx 10^{-2} M_\odot R^2$. The vortex line tension then produces an angular acceleration $\dot{\Omega}_L$ tending to speed up the crust. Numerically $\Omega/\dot{\Omega}_L \approx 3 \times 10^5 \text{ yr}$. This acceleration is large compared with that corresponding to the observed¹⁰ "wobble" in the period of the Crab pulsar; slow vortex line oscillations of some sort might be responsible for the "wobble".

A similar calculation shows that if $\Omega/\dot{\Omega} \lesssim 3 \times 10^7 \text{ years}$ ($\dot{\Omega}$ is the slowing down rate of the pulsar), vortex line tension is insufficient to prevent the lines from being stretched as the crust slows. In the slowest pulsars (those for which $\Omega/\dot{\Omega} \gtrsim 3 \times 10^7 \text{ years}$), however, the vortex line tension is sufficient to equalize the rotation rates of the crust and the neutron superfluid.

Superfluid turbulence can therefore not occur in relatively old pulsars. Furthermore, in old pulsars vortex line tension constitutes the primary interaction (if the lines are firmly pinned) between crust and superfluid. Any difference in rotation rates is damped out in the time a vortex wave takes to cross the star. This time² is vanishingly small for waves of angular frequency equal to twice the rotation frequency of the star.

(In this connexion the following numerical coincidence should be noted. Vortex waves of angular frequency $\omega > 2\Omega$, where Ω is the stellar rotation frequency, propagate with a velocity²

$$c = \frac{1}{2\pi} \sqrt{\frac{A\omega^2}{\omega - 2\Omega}}$$

where

$$A = \frac{\hbar}{2m} \ln \frac{d}{\xi}$$

For $\omega = 2\Omega$, c is infinite. For ω not much larger than 2Ω , c drops to fractions of 1 cm s^{-1} . The time for this wave to cross a neutron star can amount to months. Conceivably, then, the observed¹⁰ "wobble" in the period of the Crab pulsar may be associated with some sort of resonance in the reflexion of vortex waves. Aspects of this problem will be discussed at a later date.)

(iv) The only scatterings that may occur between neutrons and the charged particles in neutron stars take place within the normal cores of the neutron vortex lines.

Baym *et al.*⁴ have shown that the relaxation time for relative motions between charges (which determine the pulsar clock) and neutrons (which constitute the bulk of the neutron star) can approach years. This time is inversely proportional to that fraction of the neutron fluid that is not superfluid, that is, contained within the normal cores of the vortex lines (and rings). If the lines are greatly lengthened the relaxation time is proportionately shortened (it is inversely proportional to the length of line).

It is difficult to estimate the magnitude of this effect. A given line must wrap very many times about the neutron star before opposing ends come sufficiently close to reconnect and form rings. These rings can themselves survive for long times before being broken apart. It should be noted that the simple twisting of a line does not in itself contribute to the effective line length. This is because momentum can only be transferred to a vortex line normal to its length¹⁵. Therefore, only that component of line length parallel to the rotation axis is of significance; as the line twists this component is unaltered (see Fig. 1). Rings will be colliding with lines, however, exciting vortex waves in them. These waves increase the line length projected onto the rotation axis. If the line is wrapped many times about the star, so that its total length is very great, even a small amplitude wave can have a great effect. Finally, it is well known¹ that in superfluid helium vortex rings can survive for long periods of time before breaking up. They are metastable states of the superfluid. In the absence of detailed knowledge it seems safe to say that the relaxation time is decreased by this effect by several orders of magnitude.

(v) My final point is a speculative one. When an uncooked egg rotates it does so irregularly. The yolk inside moves about erratically, and in order to conserve angular momentum the rotation rate of the shell must also fluctuate. The rotating turbulent neutron superfluid must exhibit something like the same phenomenon. Vortex waves will cause the positions of vortex lines to fluctuate irregularly. In general this fluctuation must be a random, rather than a collective, motion. The velocity field, determined by the distribution of lines, will then also fluctuate. Because the line motions are random the amplitude of the angular velocity fluctuation must be very small. Undamped vortex waves have² $\omega > 2\Omega$. These waves would give rise to very rapid fluctuations in the pulsar repetition rate, and over one rotation period the effects of these fluctuations would average to nearly zero. Damped waves with $\omega < 2\Omega$ are also possible. Provided that rings collide with lines sufficiently often to excite them they could give rise to long-term random fluctuations in the pulse repetition rate.

Other fluctuations are possible. For example, in a rapidly rotating neutron star like the Crab there are $N \approx 10^{17}$ neutron vortex lines. Of these about 10^9 are "outermost", and lie directly alongside the outer crust. Their distance to the crust is $\approx 10^{-3} \text{ cm}$. Suppose their positions are fluctuating with a sufficient amplitude so that they are continually being brought against the crust and annihilated. They are also continually being regenerated to conserve the mean number of lines. This number is fluctuating over short periods of time, however. The rotation rate Ω is proportional to N . If the number of "outermost" lines is fluctuating by $\sqrt{10^9}$ then $\Delta\Omega/\Omega = \Delta N/N \approx 10^{-13}$. This is, of course, an exceedingly small fluctuation.

These considerations only apply to the neutron superfluid. The proton superfluid is constrained by the rigid magnetic field to rotate uniformly.

To summarize: the slowing down of all but the oldest pulsars (all but those for which $\Omega/\dot{\Omega} \gtrsim 3 \times 10^7 \text{ yr}$) will twist the neutron vortex lines, leading to fully developed superfluid turbulence. This turbulence drastically alters a slow oscillation of lines that might account⁹ for the "wobble"¹⁰ in the Crab pulsar repetition rate;

it increases very greatly the coupling⁴ between the neutron superfluid and the charged particles within the star; and it may conceivably lead to small random fluctuations in pulsar periods. The Crab "wobble" may also be due to resonance phenomena in the propagation of vortex waves across the star. It should be emphasized that this discussion should apply perfectly well to laboratory superfluid helium as well as neutron stars. It would be very desirable to gain insight into these phenomena by a direct experiment. Superfluid helium could be placed in a rotating spherical container so prepared that vortex lines pin effectively to the walls. The part of the charged particle system in the experiment could be played by the normal component, which will corotate with the container (the temperature should be close to T_λ to provide an appreciable quantity of normal component). Observations of the relaxation time for sudden relative motions between superfluid and container (or the attenuation of second sound) might determine the total line length. Very precise observation might reveal irregular fluctuations in the rotation rate of the container. Something like a "wobble" might be seen. Eckman pumping should commence.

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Base Sequence and Evolution of Guinea-pig α -Satellite DNA

by

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Primary structural analysis reveals that the basic repeating sequence in satellite DNA may be much shorter than reassociation studies have indicated.

THE physical properties which define the satellite DNAs of higher organisms are, first, that they are separated from the bulk of the DNA in density gradients¹⁻³ and, second, that they are able to reassociate rapidly after denaturation⁴. Taken together, these properties suggest that the satellite DNAs are composed of short base sequences, strung together in long tandem repeats⁵. Recently it has been shown that satellite DNAs are peculiar not only in their physical properties. The satellites of mouse⁶ and guinea-pig⁷ DNAs are found in the condensed fraction of isolated interphase chromatin. In mouse the sequence is found in the less soluble fraction of metaphase chromosomes⁸, and by the technique of *in situ* reassociation it is found near the centromeres⁹. For the principal fraction of the genome of all organisms there is a linear relationship between genome size and the rate of reassociation¹⁰. Using this relation the reassociation rates of mouse and guinea-pig α -satellite DNA suggest a repeat length of 300-600 (ref. 4) and 10⁵ (ref. 11) base pairs, respectively. There is also strong evidence, both from buoyant density measurements and from hybridization experiments, that these and other satellite sequences differ markedly from each other.

Clearly a knowledge of the base sequence of such fractions might both reveal underlying similarities and limit the range of their possible functions. At present, however, there are considerable difficulties in sequencing DNA, chiefly because of the lack of degradation methods comparable in specificity with the enzymes used in sequencing RNA and proteins. The one base-specific method degrades DNA to tracts containing only the

pyrimidine bases¹², and it is thereby possible to obtain the whole sequence in the degradation products if both strands are available. From this point of view, some satellite DNAs are good materials for sequencing, because the strands are easily separated in alkaline caesium chloride gradients.

Guinea-pig DNA contains at least three satellites¹¹. One of them, the α -satellite which represents about 5 per cent of the total DNA, has such a high bias in the base composition of its strands that it can be obtained as two separate strands when whole guinea-pig DNA is fractionated in an alkaline caesium chloride gradient¹³. In this article I give evidence that the α -satellite of guinea-pig DNA has been formed by the reduplication of a sequence of six base pairs, and modified by the introduction of mutations.

Evidence for a Short Heterogeneous Sequence

RNAs with simple sequences, such as the transfer and 5S RNAs, give relatively few products when degraded by enzymes specific for one or two bases¹⁴. Similarly, DNA degraded by the diphenylamine reaction yields a number of pyrimidine tracts depending on the complexity of the sequence. For example, crab satellite DNA, the simplest natural DNA, which consists mainly of repeating d(AT)¹⁵ should give mainly pTp by this reaction. More complex sequences, such as ϕ X174 DNA, give many more pyrimidine tracts¹⁶. Fingerprints of the pyrimidine tracts from the heavy (H) strand of guinea-pig α -satellite (Fig. 1) show only three strong spots (excluding inorganic phosphate), suggesting a simple sequence. By contrast the

repeat length calculated from the molar yields of some of the minor components gives values up to 2,000 (Table 1). A structure having these characteristics would be a short, repeating sequence with some sequence heterogeneity; the overall appearance of the map would be determined by the basic sequence; high values for the calculated repeat length would derive from the heterogeneity in the sequence. Clear evidence that the sequence is heterogeneous comes from observations on the melting curve and buoyant density of reassociated satellite DNA, which show that the reformed duplex is imperfectly base paired. The simplest kind of heterogeneity would be that introduced into the sequence by point mutations and in this case it would be predicted that the base sequences of the rarer tracts should be related to those of the more frequent, by one or a few base changes. Further, we might expect

base mutation would need an insertion. Sequences which cannot be derived from CCCT by one or two mutations are present in very small amounts, and together account for about 10 per cent of the total phosphate.

The relationship between pyrimidine tracts is more obvious still in the H-strand, where the most frequent sequence is TT and the next in order of frequency are T, TTT, (TTC) and (TC). The tracts in brackets were not sequenced, but irrespective of sequence, they could be derived from TT by one mutation (Fig. 3). C, left out of this list, is more likely to be introduced by transversion in the longer purine tract complementary to CCCT, and it is obvious that T could be introduced in this way, too. In the H-strand, tracts requiring more than one base mutation from TT account for 5 per cent of the total phosphate.

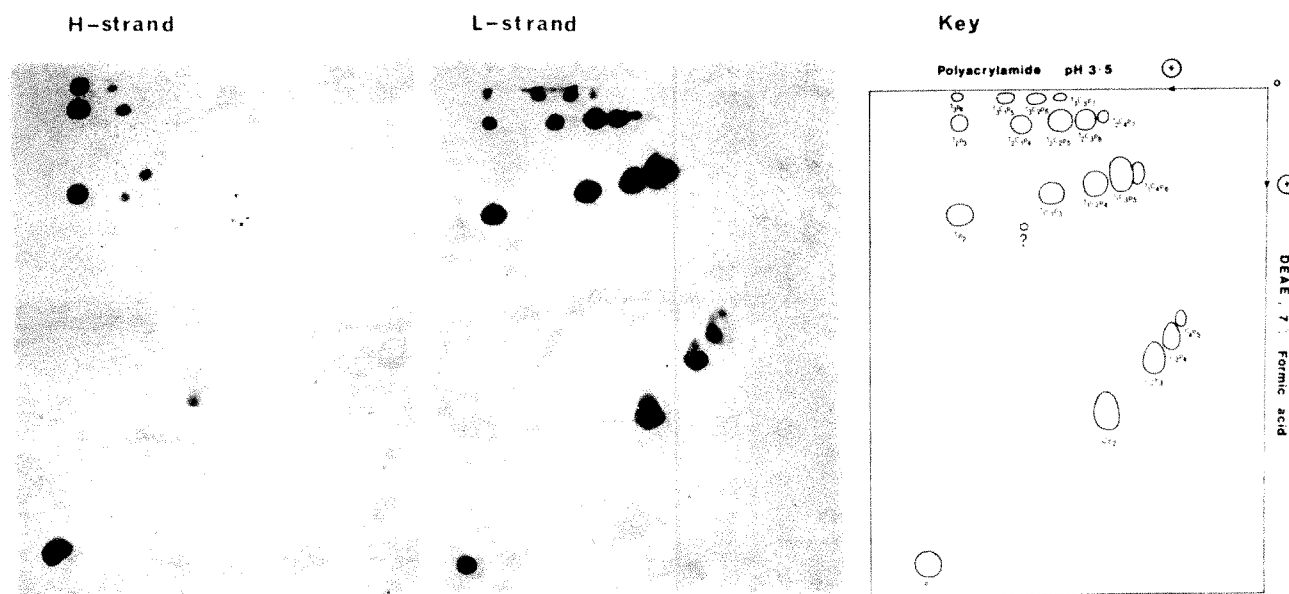


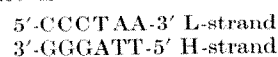
Fig. 1. Pyrimidine tracts from H and L strands of guinea-pig α -satellite. Light and heavy strands of guinea-pig α -satellite DNA, uniformly labelled with ^{32}P , were prepared as described by Flamm *et al.*¹². Caesium chloride and alkali were removed by dialysis against distilled water and the solution evaporated to dryness. The DNA was dissolved in 0.1 ml. of formic acid (66 per cent) containing diphenylamine (2 per cent) and kept at 30° C for 18 h. Water (0.1 ml.) was added and diphenylamine and formic acid were removed by extracting the solution with ether (5 \times 10 volumes). Water and residual formic acid were removed by evaporating the solution in a vacuum desiccator. The residue was dissolved in 20 μl . of 10 per cent sucrose, and applied to a polyacrylamide gel (2.3 per cent in 0.4 \times 90 cm 'Perspex' tubes) in pyridine acetate (1 M, pH 3.5). After electrophoresis (5 mA per tube) until the inorganic phosphate had moved 70 cm, the gel between the inorganic phosphate and the blue marker was squeezed out of the tubes onto a 45 \times 85 cm piece of 'DESI' paper (Reeve-Angel, Maidstone) along a line 4 cm from a short edge. The gel was allowed to dry, the paper wetted with 7 per cent formic acid, and the potential (1.5 kV) applied at right angles to the gel until the blue marker had moved about 25 cm. The paper was dried at room temperature, placed in contact with sheets of X-ray film (Kodak, 'Blue Brand') and kept in the dark for 2 weeks before developing the film. The darker spots were over-exposed to show up minor components. The spot marked with a question mark in H strand has not been identified; others were identified from their position on the paper and their composition confirmed by the analyses shown in Fig. 2.

to see the basic sequence in those tracts which occur most frequently.

The Basic Sequence

The more frequent pyrimidine tracts from the L-strand were sequenced by analysing the products from a partial degradation with snake venom phosphodiesterase, after removing the terminal phosphate residues with alkaline phosphatase, and rephosphorylating the 5'-terminus with polynucleotide kinase and γ - ^{32}P -ATP^{17,18}. The separation of the digests is shown in Fig. 2 and the main sequences are presented in Table 1. It can be seen that these sequences could all have originated from the most frequently occurring, CCCT, by one or two base changes. The purity of the sequences may be judged from the amounts of T, (CT) and (C₂T) in the partial diesterase digests of (C₃T) relative to the amounts of C, CC and CCC, for example. Perhaps the most striking feature of the sequence analysis is the presence of T at the 3' end of all sequences (except those which contain no T). Most of the sequences could be made from CCCT by either a transition or a transversion (Fig. 3). To make either CTCCT or CCTCT by a single

Taken together, the evidence suggests that the basic repeating sequence is



It is unlikely that the sequence is longer than this. An alternative repeating sequence would be



This is less probable than the first because it does not explain the higher frequency in the L-strand of CCCT compared with T, particularly in view of the four times greater chance of destroying the tetramer by mutation. Other more complex repeating sequences can be proposed, but all are internally repetitive. For example, a repeating sequence of a hundred base pairs would contain five CCCT sequences, three CT sequences, and two CCT sequences in the L-strand; and nine TT sequences and two TTT sequences in the H-strand. It seems improbable that such a sequence could arise in any way other than multiplication and divergence of a shorter sequence.

Mutations in the Basic Sequence

The fraction of mutations in the basic sequence can be calculated in two independent ways: (1) The basic repeating sequence for the L-strand CCCTAA contains no G. The present sequence contains about 4 per cent G¹³. If it is assumed that these 4 per cent have been introduced by base changes and that other base changes are equally likely, then because there are six possible base changes

base changes would be needed to reduce the CCCT from one in six to one in twenty-three, and 20 per cent would be needed to reduce TT from one in six to one in eleven bases.

Both calculations assume that all mutations were changes of one base to another: a considerable error might be introduced if a high proportion of mutations were deletions or insertions. The first calculation gives a low value because the mutations in the basic sequence are not

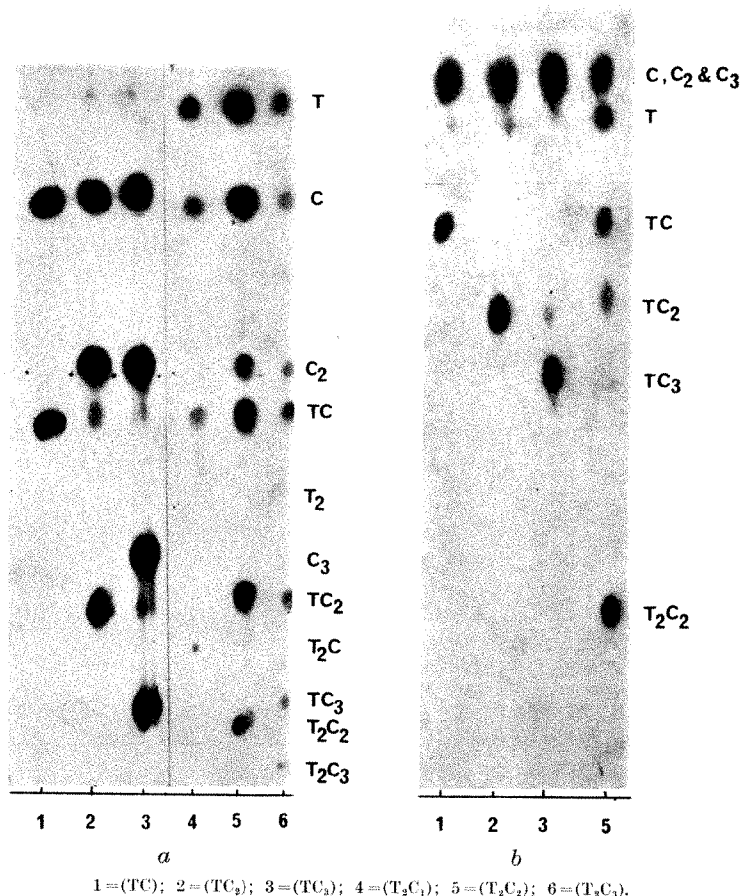


Fig. 2. Separation of the products of partial digestion with snake venom phosphodiesterase of pyrimidine tracts from guinea-pig La DNA. The pyrimidine tracts, eluted from the maps shown in Fig. 1 with 2 M triethylamine bicarbonate, were treated with alkaline phosphatase (10 μ g) at 37° C for 30 min. The solutions were dried, dissolved in 0.01 M acetic acid and applied to small flags of phosphocellulose, which were eluted with the same solvent. The solutions were dried and the residues dissolved in 20 μ l. Tris buffer (pH 7.6, 0.1 M, containing 0.015 M MgCl₂, 0.025 M mercaptoethanol + 1 μ l. of kinase fraction VI and 1 nmole γ -³²P-ATP). After 2 h at 37° C, the solutions were applied to 'DE81' paper and the phosphorylated products separated from excess ATP by electrophoresis in 7 per cent formic acid. The subsequent steps of detection and elution were as described for the original maps. The pyrimidine tracts, which now have a phosphate group at the 5' end of far higher specific activity than the uniform label, were hydrolysed with snake venom phosphodiesterase (0.01 mg/ml. in 0.02 M Tris buffer, pH 8.5). Samples removed at 10 min intervals were applied to (a) 'AE81' paper and separated by electrophoresis in pyridine acetate at pH 3.5; (b) 'DE81' and separated at pH 1.9. In cases where some of the sequences could not be resolved by this simple procedure, certain of the partial digestion products were eluted and put through a similar partial digestion and analysis. For example, T₂C₂ gives T, C, C₂, (CT) and T₂C₂ in the first analysis showing the presence of CCTT with either TCCT or CTCT or both of them. The (TC) spot gave both CT and TC, showing that all three sequences were present.

which will introduce G into the repeating sequence, three which will introduce C, four which will introduce A and five which will introduce T, the total number of base changes can be calculated as $(6/6 + 3/6 + 4/6 + 5/6) \times 4$ per cent = 12 per cent. (2) The basic repeating sequence would contain one CCCT sequence in the L-strand and one TT in the H-strand per six nucleotides. The analyses show that these frequencies in the α -satellite DNA are one in twenty-three for CCCT in the L-strand and one in eleven for TT in the H-strand. Assuming that the losses are the result of base changes in the pyrimidine tracts, or the two flanking purines, it can be shown that 23 per cent

at random frequencies; it can be seen from the base composition¹⁴ that changes from GC to AT have been more frequent than changes from AT to GC. Deviations from randomness are also clear in the amounts of the pyrimidine tracts (Table 1). On a random basis, some of the sequences should be present in equal amounts. For example, the amounts of TCCT, CTCC and CCTT, which all involve changes of C to T, should be equal. This cannot be so, because the ratio of C to T at the 5' end of (T₂C₂) is roughly one, whereas it would be two for equal amounts of the three sequences. The change from CCCT to TCCT has occurred about 10 times more frequently than

Table 1. PYRIMIDINE SEQUENCES PRESENT IN GUINEA-PIG α -SATELLITE DNA

	Pyrimidine isomer	³² P(per cent total)	Calculated repeat length	Sequences present
L-strand	P ₁	20.8	4.8	—
	C ₁	6.8	29	C
	C ₂	3.8	79	CC
	C ₃	1.2	333	CCC
	C ₄	0.3	1,667	CCCC
	T ₁	7.0	28	T
	T ₁ C ₁	8.7	34	CT
	T ₁ C ₂	8.9	45	CCT
	T ₁ C ₃	21.5	23	CCCT
	T ₁ C ₄	1.3	461	—
	T ₂	1.4	214	TT
	T ₂ C ₁	2.4	167	TCT CTT
	T ₂ C ₂	6.5	77	CCCT TCCT CTCT
	T ₂ C ₃	3.6	167	CCTCT TCCCT CTCCT
	T ₂ C ₄	0.5	1,400	—
	T ₃	0.2	2,000	TTT
	T ₃ C ₁	0.8	1,125	—
	T ₃ C ₂	1.0	600	—
	T ₃ C ₃	0.3	2,333	—
	Remaining at origin	2.9	—	—
H-strand	P ₁	34.8 ± 1.7 (4)	2.9	C
	C ₁	3.6 ± 0.2 (4)	55	T
	T ₁	11.2 ± 0.2 (4)	18	—
	T ₁ C ₁	3.4 ± 0.9 (4)	88	—
	T ₂	28.3 ± 2.5 (4)	11	TT
	T ₂ C ₁	2.8 ± 0.4 (4)	142	—
	T ₃	9.1 ± 0.6 (4)	44	TTT
	T ₃ C ₁	1.2 ± 0.4 (4)	333	—
	T ₄	2.2 ± 0.3 (3)	227	TTTT
	T ₄ C ₁	0.8 ± 0.1 (3)	750	—
	T ₅	0.9 ± 0.2 (3)	666	TTTTT

Results for the L-strand are from a single analysis on a sample containing about 60,000 c.p.m. For spots which are well separated (Fig. 1) the variance is usually less than ± 5 per cent (unpublished observations); inorganic phosphate was determined separately by electrophoresis in the second dimension only. Four analyses were done on H α -DNA; one (about 30,000 c.p.m.) was analysed as the L-strand (Fig. 1), the other three (about 3,000 c.p.m. each) by thin layer chromatography on polyethyleneimine-impregnated cellulose which separates pyrimidine isomers up to octamers (unpublished experiments). Much of the variance in these results comes from the error in counting the small amounts on the thin-layer plates; these values were included because the longer tracts were not separated by two dimensional ionophoresis. The length of a DNA sequence can be calculated from the yield of a pyrimidine tract which occurs only once in the sequence using the formula $L = 100(n+1)/x$; where $n+1$ is the number of phosphate residues in a tract containing n pyrimidines and x is the measured amount of phosphate in the tract as a percentage of the total phosphate. Tracts occurring more than once give lower values for L which should be related to the real length by a simple factor equal to the number of times that the tract occurs in the sequence. For a repeating sequence, L is the repeat length in number of bases (or base pairs for double-stranded DNA).

the change to CCCC; in a random distribution of mutations, these should be equal.

The proportion and distribution of mutations in the satellite DNA are important in relation to the possible modes of evolution of the sequence to be discussed.

Evolution of the Sequence

There is now abundant evidence that the satellite DNAs of the rodents have appeared relatively recently: none of the closely related rodents examined by Hennig and Walker¹⁹ have common satellites and the conclusion is that the sequences have arisen since these groups divided. Similar evidence for the European grasshopper²⁰ shows that this situation is not confined to the rodents. To explain the sudden appearance of millions of copies of a short sequence, representing a considerable fraction of the genome, Britten proposed²¹ that they are produced by what he has called saltatory replication, possibly in a single organism. The sequences are then imagined to spread through the species. Alternatively the sequences might accumulate slowly over a period of time comparable with the age of the species.

To produce the guinea-pig α -satellite by the saltatory process would require 10^7 copies of the basic sequence to be produced at one time. More than 10^6 mutations would then be introduced by subsequent divergence. In this case, non-random frequencies of the mutations might be taken to indicate that selection has acted to favour some mutations over others. An alternative would be to increase the sequence slowly by unequal crossing over. In this case too, the distribution of mutations should be random, in the absence of selection.

If, however, copies of the sequence were produced by a multiplication process and the periods between multi-

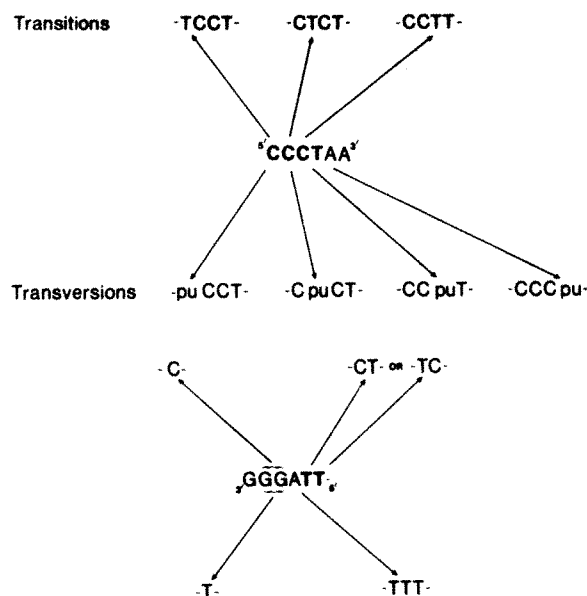


Fig. 3. Mutations in the basic sequence. The main sequences found in the H and L-strands of guinea-pig α -satellite DNA can be accounted for as shown, by transitions or transversions in the basic sequence. Alternative mutations such as deletions and insertions could account for some of the sequences.

plication steps were long enough to allow mutations to be introduced, the amount of any mutation in the final sequence would be determined by the point in the multiplication process at which it occurred: a mutation occurring early, when there were only a few copies of the sequence, would be multiplied many times and be present at a higher frequency in the final sequence than mutations occurring late, which would be multiplied less frequently. This process is explained more fully in Table 2. The possibility that the sequence might have evolved in this way disallows the conclusion that non-randomness in the proportions of mutations is a result of selection. Indeed, the presence of such a large fraction of mutations in the satellite DNA shows that the sequence is not stringently conserved, a conclusion which may be justified by contrasting the diversity of the satellite sequences with the apparent homogeneity of 5S RNA²². This RNA is the product of a repeated cistron and heterogeneity of the degree shown by the satellite would certainly have been

Table 2. PRODUCTION OF A HETEROGENEOUS REPEATED SEQUENCE BY MULTIPLICATION OF A SHORT SEQUENCE WITH MUTATIONS INTRODUCED BETWEEN MULTIPLICATION STEPS

Multiplication step	No. of bases	No. of mutations introduced between step n and $n+1$	No. of mutations accumulated
0	b	0	0
1	bm_1	bm_1x_1	bm_1x_1
2	bm_1m_2	$bm_1m_2x_2$	$bm_1m_2x_1 + bm_1m_2x_2$
3	$bm_1m_2m_3$	$bm_1m_2m_3x_3$	$bm_1m_2m_3x_1 + bm_1m_2m_3x_2 + bm_1m_2m_3x_3$
n	$b \prod_{i=1}^n m_i$		$b \prod_{i=1}^n m_i \sum_{j=1}^n x_j$

The fraction of mutations after n multiplications is

$$b \prod_{i=1}^n m_i \sum_{j=1}^n x_j / b \prod_{i=1}^n m_i = \sum_{j=1}^n x_j$$

b is the number of base pairs in the repeating sequence (six in the case of guinea-pig α -satellite). m is the factor by which the sequences are multiplied and may vary from one step to another. x is the fraction of base pairs changed in the time interval between multiplications and again may vary from one step to another; it is supposed that this fraction is the same for the whole genome. It is assumed that all the copies of the sequence, or a fraction with a typical mutation content, are multiplied at each stage; the possibility is excluded that the multiplication process selects for or against mutations. The results show, as expected, that the proportion of a mutation in the final sequence depends on the step at which it is introduced: a mutation occurring between steps 1 and 2 will be multiplied m_2 times more than one introduced between step 2 and 3. The final calculation shows that the fraction of mutations in the sequence is the same as the fraction received by the whole genome over the same period of time.

seen in sequence studies. The 5S sequence has been preserved not only among the multiple cistrons within a species but also among species separated by considerable evolutionary time. The satellite DNA must be much younger than the cumulative time between these species, for it must have arisen since the guinea-pig diverged from other rodents, and the rate of fixation of nucleotide substitutions in the sequence must have been correspondingly higher. Nevertheless, it can be shown that the number of mutations in the sequence is compatible with acceptable values for the mutation rate and the age of the satellite DNA: these are clearly interrelated. Assuming that the mutation rate for satellite DNA is the same as that of the rest of the genome, and ignoring effects of selection, it is evident that a repeated sequence produced by saltation or by unequal crossing over will receive the same fraction of mutations as the rest of the DNA over the same period of time. That this is also true for a repeated sequence produced by slow multiplication is shown by the calculation in Table 2.

The mutation rate has been calculated from the rate of fixation of amino-acid substitutions, presumed to be neutral, in the fibrinopeptides²⁴ as around 4.2×10^{-9} changes per base pair per year. At this rate, $0.2/4.2 \times 10^{-9}$ or about 5×10^7 years would be needed to change 20 per cent of the bases in satellite DNA. The divergence point of guinea-pig from other rodent species has not been fixed precisely, but it could have occurred as long ago as 50 million years²⁵.

Conclusion

The description of guinea-pig α -satellite DNA given by sequence analysis is somewhat different from that derived from the kinetics of renaturation which suggest a repeating sequence of around 10^5 base pairs¹¹. It is likely that the difference is a consequence of the high proportion of mutations in the sequence: a similar, though smaller, discrepancy is found for mouse satellite DNA (unpublished results of A. Carr-Brown, E. M. S. and P. M. B. Walker). This is the first indication that mismatching might have an effect on the rates of reassociation of nucleic acids and it should be borne in mind that all repeated fractions of higher organism DNA give reformed duplexes which are badly matched. It is possible that the rate of reassociation gives a gross over-estimate of the length of the repeating sequence in these cases, too.

The primary structure of the basic repeating sequence seems to rule out the possibility that the DNA codes for a protein. Of the six possible reading frames for the two strands, two give alternating nonsense codons; the other

reading frames could give repeating dipeptides of Pro-Asn, Leu-Thr, Arg-Val, or Gly-Leu.

So far we have been unable to find any relationship between the sequence of the guinea-pig α -satellite DNA and the light satellite of mouse DNA. The latter seems to derive from the repetition and mutation of a somewhat longer sequence (between eight and thirteen base pairs) which includes the sequence of six pyrimidines TTTTTC.

A simple structure for satellite DNA explains other properties which were formerly puzzling. The bias in base composition, especially between strands is expected of a simple sequence. Melting temperatures and buoyant densities different from those predicted from the base composition are expected of a simple sequence by analogy with the properties of simple synthetic polymers, which differ considerably from those of complex sequences^{26,27}.

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Requirement of Serum for DNA Synthesis in BHK 21 Cells: Effects of Density, Suspension and Virus Transformation

by

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Cell density, attachment and serum concentration affect initiation of events leading to DNA synthesis in normal cells. Transformed cells are less restricted.

THE growth of fibroblasts in culture is inhibited when the cultures reach a characteristic saturation density¹⁻³, when growth is arrested in the G1 phase of the cell cycle^{4,5}. Similarly, the growth of fibroblasts is also dependent on anchorage⁶—they will not grow in suspension or in soft gel, for example—and on serum⁷⁻⁹, the addition of which can even stimulate dense static cultures to grow². The

characteristics of fibroblasts transformed by tumour viruses are, however, quite different: they continue to grow even in cultures too dense for normal cells¹¹⁻¹⁴; they may continue to grow even in suspension^{15,16} or even, to some extent at least, in the absence of serum^{8,10}.

We have studied the serum requirement of both normal and transformed cells in different conditions of

density and anchorage in the hope of finding a common explanation for all three factors and a reason why infection with a tumour virus should alter these characteristics. Our findings, together with those of Dulbecco¹⁷ (following article), throw light on the events which lead to DNA synthesis.

We have used the BHK 21 hamster cell line (C13 clone) and a polyoma virus transformed derivative (PyJ). The cells were grown in Dulbecco's modified Eagle's medium containing 10 per cent calf serum. The proportion of cells incorporating DNA was measured in plastic Petri dish cultures by counting labelled nuclei after exposure to ³H-thymidine (10 μ Ci) for 20 h, extraction with 5 per cent trichloroacetic acid and overlay with stripping film for 48–72 h. The incubation period involves only the first cycle of DNA synthesis. Neither reducing the concentration of ³H-thymidine sixteen-fold nor adding a nine-fold excess of unlabelled thymidine affected the percentage of labelled nuclei. The calf serum came from large pretested batches stored at -10°C and was used unheated.

We obtained resting cells in G1 phase and depleted of serum factor, by incubating Petri dish cultures containing 3×10^5 cells for 2–3 days in 0.5 per cent calf serum¹⁰ which was then replaced by serum-free medium. Because, after the addition of ³H-thymidine and incubation for a further 20 h, there were very few radioactive nuclei (0.1 per cent), we used this procedure in all our experiments.

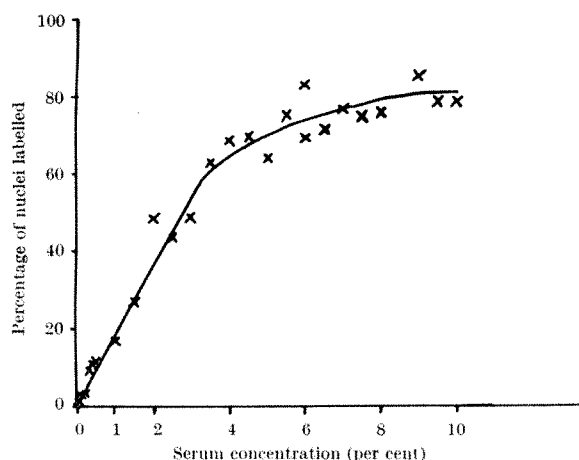


Fig. 1. Response of BHK 21 cells to serum concentration in layer culture. Resting BHK 21 cells (3×10^5 per dish) were incubated for 20 h with 10 μ Ci of 23.5 Ci/mmol [methyl-³H] thymidine and increasing serum concentrations. The cultures were then washed three times with Tris buffer (pH 7.4), twice with 5 per cent trichloroacetic acid at 4°C , twice with ethanol, and exposed for 72 h for autoradiography. Labelled nuclei were counted in a minimum of 300 total cells.

Increasing concentrations of serum were added to the resting cultures and at least 300 radioactive nuclei were counted in each experiment (Fig. 1). The percentage of cells incorporating thymidine increases linearly up to about 50 per cent with 3 per cent serum; thereafter the curve flattens, with 80 per cent of the cells labelled in 10 per cent serum. There was some variable cell loss (up to 30 per cent) in the complete absence of serum, but total cell numbers varied little with increasing serum concentration. Although this agrees with Temin's report of a linear relationship for chick fibroblasts initiated by serum¹⁹, we found variations in the curve in different experiments, so that we do not claim a reproducible first order interaction.

Because most nuclei were either heavily labelled or unlabelled at all serum concentrations, it is unlikely that this procedure is measuring variations in the uptake of thymidine.

To investigate the effect of suspension on the response to serum, we suspended resting BHK 21 cells in medium containing 1 per cent 'Methocel' (4,000 centipoises, Dow

Chemical Corporation) and ³H-thymidine at various serum concentrations. After incubation for 20 h in 1 ml. volumes above a solid medium containing 0.5 per cent serum and 0.9 per cent agar (Difco Bacto), each culture in 'Methocel' medium was washed into a Petri dish with 4 ml. of fluid medium containing 5 per cent calf serum and 10^{-5} M unlabelled thymidine and allowed to spread. Autoradiographic counts of the labelled nuclei showed that DNA synthesis was again initiated in proportion to serum concentration. The slope of the curve in Fig. 2, however, indicates that suspended cells are about sixty times less sensitive than anchored cells, so that even 50 per cent serum only initiated DNA synthesis in 12 per cent of the cells. This effect was not caused by the suspending medium because cells anchored in the presence of 'Methocel' medium showed the usual sensitivity of layer cultures.

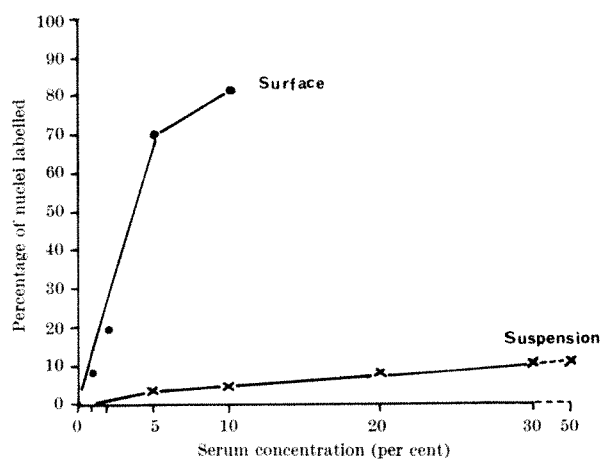


Fig. 2. Response of BHK 21 cells to serum concentration suspension and layer culture. Layer cultures were treated as in Fig. 1; suspension cultures are of 2×10^5 BHK 21 cells in 1 ml. medium containing 1 per cent 'Methocel', ³H-thymidine (2 μ Ci) and increasing serum concentrations. After 20 h at 37°C each suspension was diluted in 4 ml. of 5 per cent serum medium containing 10^{-5} M unlabelled thymidine and incubated in plastic Petri dishes for 5 h to allow spreading. Dishes were then treated as layer cultures for autoradiography.

Suspended BHK 21 cells exposed to high concentrations of serum not only initiate DNA synthesis, but also grow. When these cells were seeded in agar suspension cultures containing 50 per cent serum, 30 per cent of the cells formed small colonies within 7 days.

Cell Density, Wounding and Serum Response

We exposed layers of resting cells originally seeded at different densities to increasing serum concentrations. The proportion of cells synthesizing DNA (Fig. 3) reveals that the serum requirement per incorporating cell increases with the density: in other words, the sensitivity of the cells diminishes. Thus even BHK 21 cells exhibit density dependent inhibition in low concentrations of serum. In standard medium containing 10 per cent serum, the effect cannot be observed because nearly all cells are incorporating even at high densities. Fig. 4 shows the effect of final cell density on the response to a fixed serum dose. The response is greatest at about 5×10^5 cells per dish. Above this density the effect of density dependent inhibition is seen. The reduced response at lower densities is probably caused by lack of conditioning factor(s).

Changes in cell density and topography which lead to DNA synthesis may be produced by wounding dense sheets of cells so that emigration from the edge takes place^{2,18}. To examine the response to serum factor of the emigrating cells, resting cultures at a density of 10^6 per dish were wounded by removing a 10 mm strip of cells with a silicone rubber wedge; the detached cells were removed by washing with serum-free medium, and the response to increasing doses of serum was examined as

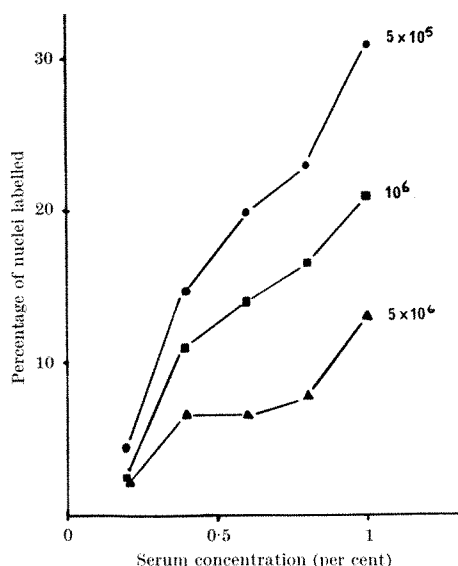


Fig. 3. Effect of cell density on the response of BHK 21 cells to serum in layer culture. Cells were plated at different densities as shown, incubated, and prepared for autoradiography as in Fig. 1.

before. In the absence of serum very few cells moved from the layer to the bare area, but as the concentration of serum was increased, the number of migrating cells also increased. We therefore conclude that serum contains a factor which stimulates movement. Compared with cells in an undisturbed layer, the initiation of DNA synthesis in emigrating cells is highly sensitive to serum factor (Fig. 5). In the emigrating zone the proportion of cells labelled is even higher than would be expected in standard cultures of approximately equivalent density (2 to 5×10^5 per dish).

The stimulation of DNA synthesis in chicken cells in monolayer culture by serum can also be mimicked by insulin at high concentrations¹⁹; insulin also stimulates BHK 21 cells to form colonies in agar containing 10 per cent serum (personal communication from Dr I. Macpherson). When serum titrations were done in layer cultures to which insulin (23.1 U per mg, Allen and Hanbury) had been added at 10 μ g per ml. (Fig. 6), we found that the action of low concentrations of serum was potentiated by the hormone but that there was no effect in serum-free media.

Polynoma Transformed BHK 21 Cells

Fig. 7 shows the behaviour of PyJ cells from 2 day layer cultures in 0.5 per cent serum when used for titrations in layer and suspension culture. DNA synthesis occurred in at least half the cells in both layer and suspension, even in serum-free media. Moreover, near maximum response was observed at very low serum concentration (0.4 per cent). Not only do these transformed cells respond more readily to serum, but response is hardly affected by anchorage.

Because the transformed cells incorporated ³H-thymidine in the complete absence of added serum, we had to determine the contribution of the 2 day period in 0.5 per cent serum medium which was used to obtain resting cultures. We found that the transformed cells could not be maintained as layer cultures in concentrations of less than 0.5 per cent for prolonged periods because they detached from the surface. Accordingly, transformed cells from standard cultures were maintained in suspension in serum-free 'Methocel' medium for 4 days. When ³H-thymidine was added for only 3 h to these cultures, about 50 per cent of the cells were labelled. It seems therefore that DNA synthesis can continue long after the cells have been removed from media containing serum, but

that the transformed cells will not divide indefinitely in the absence of serum. The number of cells in both layer and suspension culture in serum-free media fell steadily in spite of the continuously high proportion incorporating thymidine, until no viable cells remained. Thus few, if any, transformed cells are prevented from entering the S phase by the lack of added serum factor, but although they continue to synthesize DNA they may die subsequently before entering another cycle.

Serum Requirement for DNA Synthesis

Our results show that serum induces DNA synthesis in BHK 21 cells in any of the states examined, including suspension, and the number of cells in which this occurs is a function of the serum dose. The probability of response—that is, the slope of the dose response curve—was, however, markedly affected by the state of the cells. Suspended cells were about sixty times less sensitive than cells attached to a surface, while the attached cells showed a three-fold reduction in sensitivity when the density was increased ten-fold. Thus BHK 21 cells are sensitive to

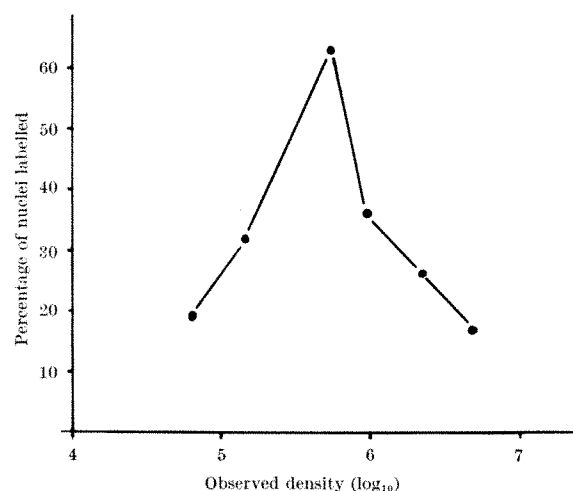


Fig. 4. Effect of cell density on the response of BHK 21 cells to 1 per cent serum in layer culture. The abscissa shows the density of cells as counted at termination of the experiment.

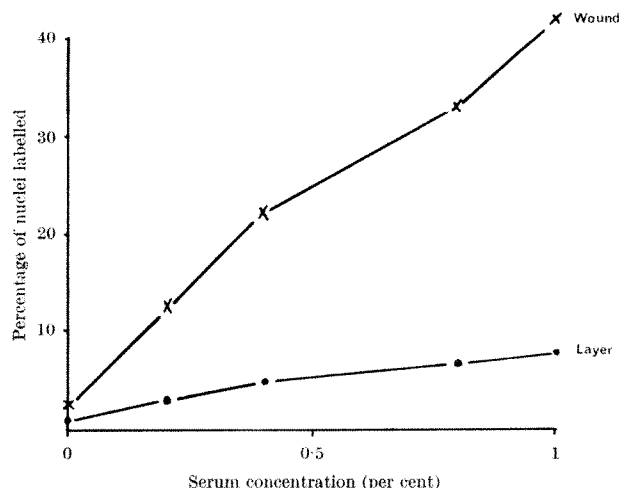


Fig. 5. Response to serum concentration of BHK 21 cells in undisturbed layer and wound edge in same cultures. Resting cultures of 10^6 BHK 21 cells per dish were wounded by wiping a 10 mm strip free of cells with silicone rubber, washed with Eagle's medium and incubated for autoradiography as described in Fig. 1. Counts of the per cent of labelled nuclei were made in a cell layer remote from, and at the edge of, the wound.

density dependent inhibition although the effect, which can be detected only at low serum concentration, is clearly less marked than that shown by many other cell types.

Cells migrating at lower density along the edge of a wounded layer are even more sensitive to serum factor than would be predicted from this reduced density, and we believe that this is caused by a diffusible conditioning factor (unpublished observations). Moreover, the dose response curves for normal cells, although differing in slope, pass through the origin. There is therefore an absolute requirement for serum factor if DNA synthesis is to be initiated, even in situations in which cells respond very readily, such as the edge of wounds.

The increased sensitivity of BHK 21 cells to serum in the presence of insulin cannot be reproduced by physiological concentrations of the hormone; the effect could therefore be caused by an impurity.

As the density of a population of cells increases, the requirement for serum factors of any individual cell in the population before it can start DNA synthesis also increases. The rate of change of this serum requirement, which is characteristic for any cell type, clearly limits the final density to which a population of cells can grow at given serum concentration. The saturation density can be correspondingly modified by increasing or decreasing the serum concentration. But the serum factor concentration in a culture is not likely to be constant, as it will depend on removal or destruction by the cells and on medium changes. The final density of a culture will therefore be a complex function involving many factors. The reason for the change in response to serum which accompanies alterations in shape, attachment to a surface and interaction with other cells cannot at present be explained. Dulbecco¹⁷ concludes that cell topography can affect entry to DNA synthesis independently of serum, but that serum can compensate for this action. Our results with BHK cells do not lead directly to this conclusion, but they are not incompatible with it.

In several respects, virus transformed cells differ from normal cells in their response to serum. First, the initial response is very high because a large proportion of cells are synthesizing DNA even in 0.2 per cent serum. Second, in over half the transformed cells, DNA synthesis takes place in the complete absence of added serum. Finally, there is little difference between anchored and suspended cells. Either the requirement for exogenous serum factor is removed or greatly reduced, or the cell can retain the factor (or its products) from earlier propagation. We have not yet been able to distinguish between these two possibilities, because in spite of their ability to initiate

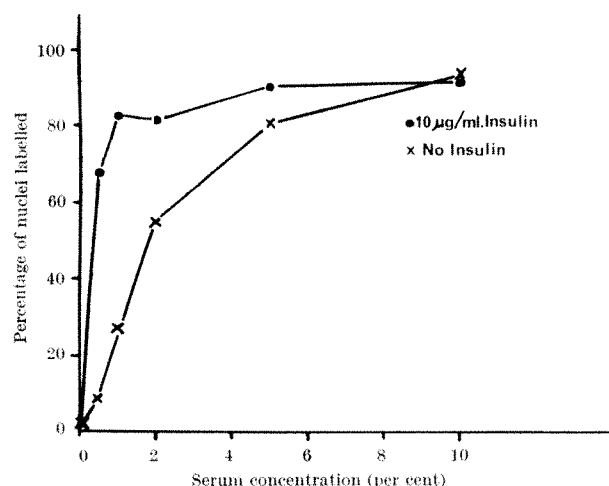


Fig. 6. Effect of insulin on the response of BHK 21 cells to serum in layer culture. Layer cultures were treated as in Fig. 1, with and without insulin (10 μ g/ml.).

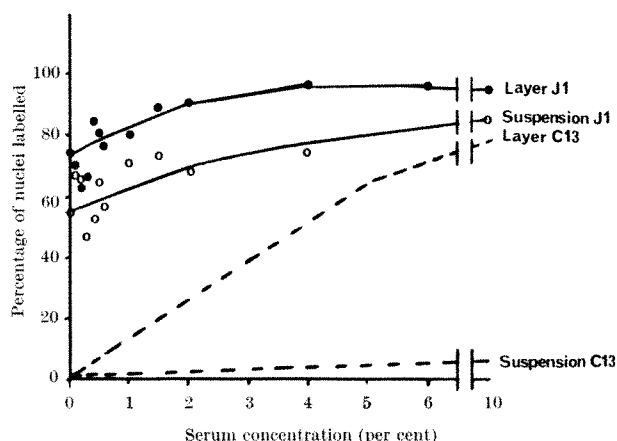


Fig. 7. Response of BHK 21/PyJ cells to serum concentration in layer and suspension culture. 3×10^5 PyJ cells per layer culture were incubated for 2 days in 0.5 per cent serum medium and used for serum titrations as in Fig. 1; suspension cells were treated as in Fig. 2. Broken lines indicate a comparable response of normal BHK 21 cells from Fig. 2.

DNA synthesis, a population of transformed cells cannot maintain itself in the absence of serum. Other classes of serum factor must therefore be required for maintenance²⁰ and for the full growth cycle of cells, whether normal or transformed.

These additional factors are presumably not limiting in medium containing serum, so the ability of transformed cells and the failure of normal cells to multiply in conventional medium, at high density, and in suspension, could be ascribed simply to the differences in requirement for the factor needed for DNA synthesis. In these culture conditions, normal cells are very insensitive to this serum factor, so the cell cycle cannot be initiated. Transformed cells, however, escape the restriction because their ability to initiate DNA synthesis is partially or completely independent of this factor. We do not know whether or not the elimination of the requirement of serum factor for initiation of DNA synthesis is a primary change effected by the addition of the viral genome to the cell. The number of possible viral functions is limited, and it is tempting to suppose that only one or two gene products are ultimately responsible for all the changes in cell physiology such as movement, orientation, surface components and carbohydrate metabolism, as well as increased growth capacity. The removal of the serum factor requirement could be linked to any or all of these changes.

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Topoinhibition and Serum Requirement of Transformed and Untransformed Cells

by

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The multiplication of tissue culture cells is controlled by several different physiological characteristics, the quantities of which can be measured. Transformed cells are characterized by the values of some of these physiological parameters.

WHEN a confluent culture of 3T3 cells on a plastic Petri dish, in which most cells do not synthesize DNA, is wounded, DNA synthesis is initiated in the cells that migrate into the denuded area^{1,2}. Although this initiation depends in part on the release of cells from extensive contacts with other cells, which seem to inhibit DNA synthesis (topoinhibition, TI), it is clear that other factors are also involved². To study these other factors and their relationships to TI, I have examined the effect of serum, which is required for the growth of many cells, on the initiation of DNA synthesis in cells in the wound and in the cell layer far from the wound, and on other cellular phenomena, using a variety of cell types. The main results presented here are a separation of the effects of serum deficiency and topoinhibition, and the derivation of new criteria for characterizing virus-transformed cells.

The following permanent lines were used for cell cultures: 3T3³, originally from Dr H. Green; BALB/c-3T3⁴, from Dr G. J. Todaro; BHK, a derivative of BHK 21, Cl13⁵, originally from Dr M. G. P. Stoker; BSC-1⁶, a line of green monkey kidney cells; SV3T3⁷, an SV40-transformed line of 3T3 cells, originally from Dr H. Green; PyS4, a polyoma-transformed line of BHK cells produced in this laboratory; H50⁸; SV FL101, a "flat" variant of SV3T3 cells⁹; Cl 32-5 and Cl 41-1, two similar variants obtained in this laboratory from SV3T3 cells. For short-term cultures we used ME, secondary mouse embryo cultures; HaE, secondary hamster embryo cultures; BMK, primary baby mouse kidney cultures.

Cultures were prepared on Nunc plastic Petri dishes in reinforced Eagle's medium containing 10 per cent calf serum, except for BHK cultures, which also received 10 per cent TP (TP = 3 per cent Difco tryptose phosphate in water); BSC-1 cultures, which were grown in medium containing 10 per cent foetal calf serum and 5 per cent TP, and for BMK cell cultures, which had 10 per cent horse serum instead of calf serum. Specific treatments are indicated in the legends to the figures.

Wounding, labelling and autoradiography were carried out as already described². Various regimes of pretreatment were used, and Fig. 1 describes these graphically.

Effect of Serum Concentration on Initiation of DNA Synthesis

Confluent cultures of 3T3, BALB/3T3 or BHK cells were washed with serum-free medium and then covered with medium containing variable amounts of serum (regime B, Fig. 1). Figs. 2A and B illustrate the results. They reveal two features: (1) the proportion of DNA-synthesizing cells in the wound is much higher than in the layer; (2) the ratio varies at different serum concentrations. At low serum concentrations, incorporation in the wound is extremely sensitive to variations in serum concentration. This behaviour affords a sensitive way to measure low serum concentrations (from about 10⁻⁴ upwards).

The proportions of cells in the wound and in the layer

which synthesize DNA are strongly affected by the treatment of the cells before wounding. First, pre-exhaustion of factors in the medium by long incubation before wounding (regime A, Fig. 1, without changing the old medium at or after wounding) markedly reduces the number of DNA synthesizing cells in the layer, but has little effect on incorporation in the wound (Table 1). The exhaustion probably affects serum factors because, after one day's exhaustion, the proportion of radioactive nuclei in the layer is very roughly proportional to the initial serum concentration. Second, cultures which were washed when serum factors were still abundant (regime B, Fig. 1) also display high incorporation in the wound in the absence of added serum. If, however, in similarly treated cultures, the serum factors were mostly exhausted at the time of washing, there was very little incorporation in the wound without further serum addition. Third, cultures of BALB/c-3T3 treated according to regime C, Fig. 1, sometimes show a higher proportion of DNA-synthesizing cells in the layer than when phase 3 (incubation in serum free medium) was omitted. The effect, which is only noticeable in cultures to which serum was added in phase 4, is, however, not always reproducible; neither is it clearly shown by other cell lines.

The first effect shows that the serum factors which are required to initiate DNA synthesis in the layer are exhausted more rapidly than the factors which are required to initiate DNA synthesis in the wound; it is not known whether the two types of factors are identical. The second effect shows that some factors have a persistent action, which continues after they have been removed from the medium; they may stick to the cells or cause a lasting cell modification. The third and only occasional effect may perhaps be caused by release from the cells of factors which are able to stimulate the initiation of DNA

A	1 10 per cent calf	2 Medium	3 Test		
	Medium	Various serum conc.			
B	1 10 per cent calf	2 Wash	3 Medium	4 Test	
	Medium	SFM	Various serum conc.		
C	1 10 per cent calf	2 Wash	3 Incubate	4 Medium	5 Test
	Medium	SFM	SFM	Various serum conc.	

Fig. 1. Pretreatment regimes. SFM, serum-free medium; Test, wounding, labelling with ³H-thymidine between 8 and 32 h after wounding, followed by fixation and autoradiography.

synthesis, especially in the layer, but require serum for their action. BHK cells have been shown to produce factors which stimulate DNA synthesis in the presence of serum¹⁰.

The very strong influence of the cell pretreatment may cause variability in experiments conducted in otherwise similar ways, if the pretreatment regime is not closely controlled.

We have extended this approach to a variety of cultures of either transformed or untransformed cells. The results show four types of behaviour, as shown in Figs. 2 and 3.

First, with 3T3, BALB/c-3T3 and BHK cells, the two curves (wound and layer) begin close to the origin, indicating that the initiation of DNA synthesis strongly requires serum, both in the layer and in the wound (Fig. 2, A and B). The ratio of pairs of values of the two curves corresponding to the same serum concentration has its highest value near the origin and then declines as serum concentration is increased.

Table 1. EFFECT OF SERUM EXHAUSTION ON INITIATION OF DNA SYNTHESIS IN CULTURES OF 3T3 CELLS

Serum concentration per cent	Days of incubation before wounding	Cell concentration in 100 × 450 μ fields		Proportion of radioactive nuclei		Prop. rad. nuclei wound/Prop. rad. nuclei layer
		Layer	Wound area of maximum labelling	Layer	Wound area of maximum labelling	
2	1	21.6	12.8	0.008	0.20	25
	2	18.1	7.1	0.006	0.58	97
	3	17.8	4.0	0.004	0.53	132
10	1	38.7	23.2	0.091	0.39	4.3
	2	31.8	12	0.030	0.51	17
	3	28.8	11.5	0.015	0.36	22
20	1	41.3	19.4	0.170	0.50	3.0
	2	49.8	5.0*	0.037	0.70	19
	3	64	14.6	0.006	0.42	70

* This wound was wider than the others.

Cultures were started with 2.5×10^5 cells per 50 mm Petri dish in 10 per cent calf serum medium. After 24 h, the culture medium was changed to one containing the indicated amount of serum. At subsequent times, some cultures were wounded, and after 22–24 h ^3H -thymidine was added at the final concentration of 7×10^{-7} M, 1.2 $\mu\text{Ci/ml}$. After labelling for 6–7 h, the cells were fixed and autoradiographed. Times are measured from the medium change.

Second, ME cultures kept for several days in low serum and then tested behave similarly, but an especially high proportion of the wound cells can synthesize DNA in the absence of serum (Fig. 3A). The proportion is further increased by addition of serum. The wound and layer curves have the same general relationship to each other as in the previous case. Similar results are obtained in HaE cultures.

Third, in cultures of epithelial cells, such as BMK or BSC-1, a considerable proportion of the cells can synthesize DNA in the absence of serum, especially in the wound, and the proportion remains nearly constant as serum concentration is increased, so that the ratio of the proportion of labelled cells in the wound to that in the layer does not change appreciably with serum concentration (Fig. 3B).

Fourth, in cultures of transformed cells, the proportion of DNA-synthesizing cells is either independent of serum concentration or is only slightly dependent, becoming maximal at low concentrations (1 per cent or less); furthermore, there is very little or no difference between the wound and the layer.

Serum Requirements for other Cellular Functions

A requirement for cell survival is demonstrated by the effect of prolonged serum starvation. For most cell types, both untransformed and transformed, the proportion of incorporating cells (irrespective of serum concentration) is higher if they come from a serum-containing culture than from a serum-starved culture. In the absence of serum, death is observed within a day or two, with all fibroblastic cell types, both transformed and untransformed, as shown by progressive cell detachment and accumulation of dead cells in the medium. In cultures in

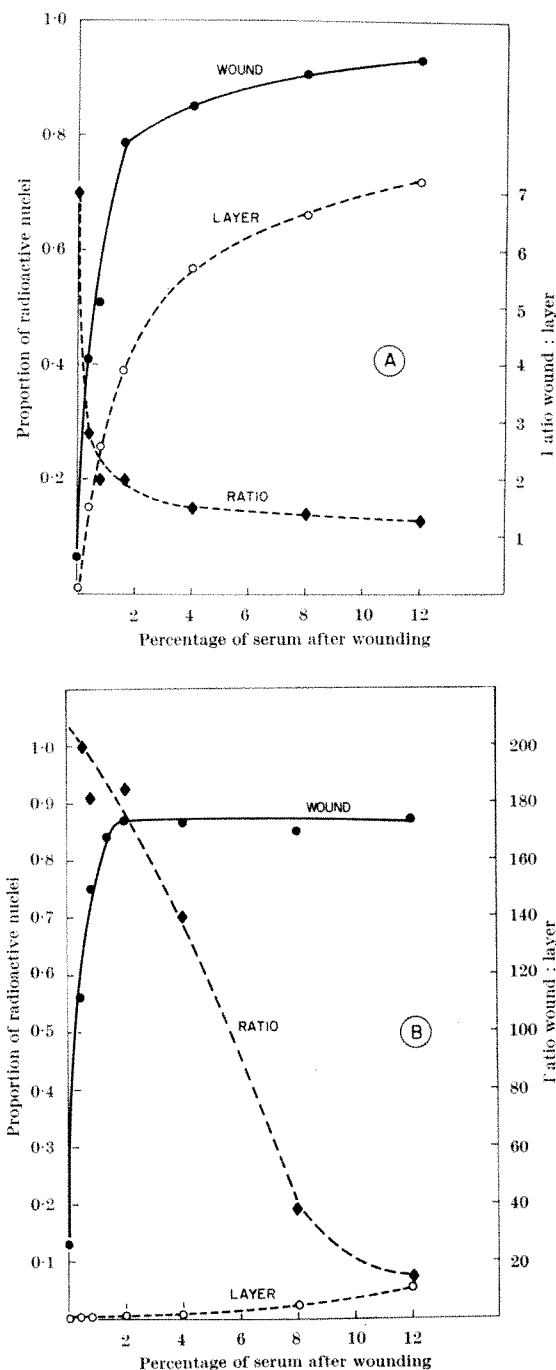


Fig. 2. Dependence of proportions of radioactive nuclei on serum concentration. Confluent cultures were treated according to regime B, Fig. 1. Labelling was done with ^3H -thymidine, 1.2 $\mu\text{Ci/ml}$, 7×10^{-7} M. A, BHK cells; B, BALB/c-3T3 cells.

which cell multiplication in the layer is blocked by topoinhibition (for example, BALB/c-3T3 cells) the number of attached and morphologically normal cells remaining after a given time is proportional to the initial serum concentration (Fig. 4).

The proportion of cells in mitosis is greatly affected by the conditions of the cultures. In cells showing strong TI (for example, BALB/c-3T3 or 3T3), mitoses are absent or very scarce at any serum concentration. In transformed cell cultures, the proportion of mitotic cells both in the wound and layer depends on the serum concentration. In SV3T3 cell cultures, tested under regime B, Fig. 1, nearly 100 per cent of the cells synthesized DNA, both in the wound and in the layer, at any concentration of

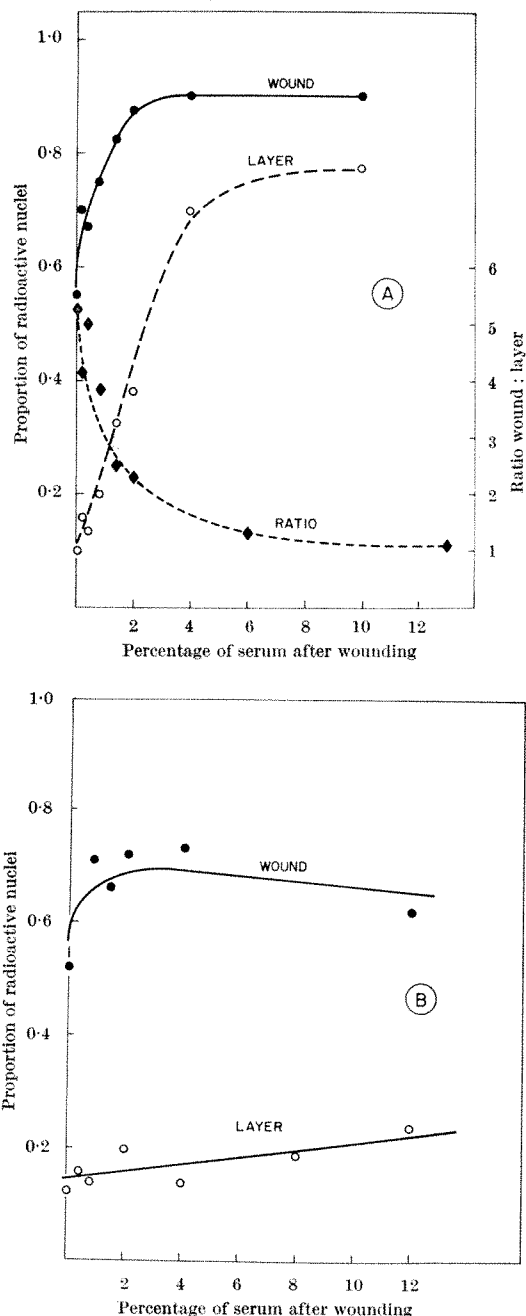


Fig. 3. Dependence of proportions of radioactive nuclei on serum concentration. A, ME cells, secondary cultures prepared with 5×10^6 per 50 mm dish in 10 per cent calf serum medium. After a day incubation they were changed to 0.5 per cent calf serum medium and left in that medium for five days; they were then treated according to regime B, Fig. 1. B, BSC-1 cells, treated according to regime B, Fig. 1.

serum, but the total number of cells entering mitosis depended markedly on serum concentration (Table 2). The ability of cells that have initiated DNA synthesis to undergo mitosis therefore still depends on the serum concentration, which may explain the much more pronounced dependence of transformed cells on serum concentration for growth than for initiation of DNA synthesis (personal communication from R. Holley).

Conditions for the Initiation of DNA Synthesis

The different behaviour of the various cell lines with respect to initiation of DNA synthesis can be described by the following three parameters (Fig. 5):

Table 2. RATES OF MITOSIS IN SV3T3 CELL CULTURES AT DIFFERENT SERUM CONCENTRATIONS

Hours with colcemid (from time zero)	No serum	Serum concentrations		
		1 per cent	4 per cent	8 per cent
7-23	0.010	0.020	0.021	0.024
23-30	0.004	0.034	0.030	0.037
30-47	0.0008	0.012	0.025	0.023

At time zero parallel cultures were washed with serum-free medium, then overlaid with 5 ml. medium containing various concentrations of foetal calf serum and incubated at 37° C. At the indicated times colcemid was added to some cultures (final concentration 0.06 μ g/ml.); the cultures were returned to the incubator; at subsequent times the cultures were washed with saline, kept 5 min in hypotonic saline (physiological saline 1:4 in water), dried, fixed and stained with Giemsa. Mitotic and non-mitotic cells were counted under oil immersion. Numbers indicate proportion of cells entering mitoses per hour. Proportions of radioactive nuclei in parallel cultures labelled with 3 H-thymidine between 7 and 30 h were 99 per cent or higher irrespective of serum concentration.

(1) The serum requirement in the wound (WSR), defined as $\frac{MxWP - MnWP}{MxWP}$ where MxWP is the highest proportion of radioactive cells in the wound at high serum concentration, MnWP is the proportion of labelled cells in the wound without serum. Because the wound cells are maximally sensitive to serum, WSR represents the true serum requirement of the cells, when other conditions affecting DNA synthesis are removed.

(2) Topoinhibition (TI), that is, topographical factors inhibiting DNA synthesis in the layer². TI is defined as the ratio $\frac{MnWP - MnLP}{MnWP}$, where MnLP is the proportion of incorporating cells in the layer at or near zero serum concentration where the ratio is maximal.

(3) Compensation of topoinhibition by serum (CTI) is defined as the ratio $\frac{TI - TIhs}{TI}$, in which TIhs is topoinhibition at high serum concentration (about 10 per cent in these experiments), where it is little affected by small changes of serum concentration ($TIhs = \frac{MxWP - MxLP}{MxWP}$).

CTI is essentially zero in cultures of epithelial cells and is unmeasurable in those of transformed cells. CTI may be affected by cell-released factors, as is sometimes the case for BALB/c-3T3 cells.

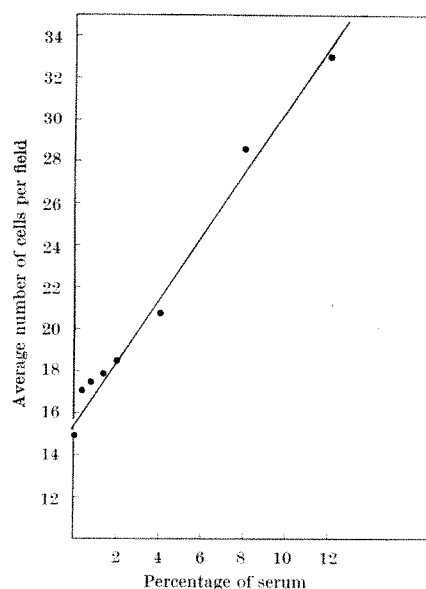


Fig. 4. Numbers of cells per microscopic field in cultures of BALB/c-3T3 cells after exposure to various serum concentrations. Confluent cultures were washed with serum-free medium and then covered with 5 ml. of medium containing various concentrations of calf serum. 32 h later the cultures were washed with saline, fixed with 5 per cent trichloroacetic acid, rinsed several times with ethanol and stained with Giemsa. Nuclei were counted using a $\times 100$ oil immersion objective.

Table 3. PHYSIOLOGICAL PARAMETERS CONCERNING INITIATION OF DNA SYNTHESIS IN DIFFERENT CELL TYPES

Cell types	Length of preincubation in SFM	WSR	TI	CTI
Untransformed cell types				
BHK	None	(a) 1.0 (b) 0.80 (c) 0.92	0.75 0.90 0.85	0.46 0.87 0.62
BALB/c-3T3	None	(a) 0.85 (b) 0.72	1.0 1.0	0.05 0.025
3T3	2 days	0.82	1.0	0.80
ME	None	0.73	0.81	0.21
	4 days in 0.5 per cent serum medium	0.40	0.80	0.82
BSC-1	None	0	0.79	0
BMK (epithelial cells exclusively)	None	0	0.92	0
Transformed cell types				
SV3T3	None	(a) 0 (b) 0.27	0 0.17	UM UM
	2 days	0 0.27	0 0	UM UM
PyS4	None	(a) 0.18 (b) 0.27	0 0	UM UM
"Flat" revertants				
SV FL 101	None	0.01	0.12	UM
CI 32-5	None	0.18	0.12	UM
CI 41-1	None	0.29	0.45	1.0

UM = Unmeasurable.

(a), (b), (c) indicate repetition of the same experiment.

The three parameters for various cell types are given in Table 3 and are plotted in the correlation diagram of Fig. 6. The diagram shows that WSR, TI and CTI are not obviously related.

The independence of WSR and TI is also indicated by the different effects of pretreatments on DNA incorporation in the layer and in the wound. Partial exhaustion of serum factors strongly affects labelling in the layer (but not in the wound); and cells washed from unexhausted medium retain a memory of the serum factors with resulting high incorporation in the wound (but not in the layer). Other pretreatment regimes (see Table 3) markedly enhance incorporation in layers of BALB/c-3T3 cell cultures but little in wounds. It is therefore likely that TI does not derive from the occlusion of cell receptors for serum factors in crowded layers², but is the consequence of cell interactions that directly affect DNA synthesis. TI is therefore identifiable with contact inhibition of growth, often postulated as a control mechanism of cell multiplication, independently of serum factors.

Different Functions of Serum

At least four functions are indicated by my results, two of which are concerned with the initiation of DNA synthesis. Of these, one is a direct role and is measured by WSR; the other is to counteract TI in cultures of fibroblastic cell types displaying TI (but not in cultures

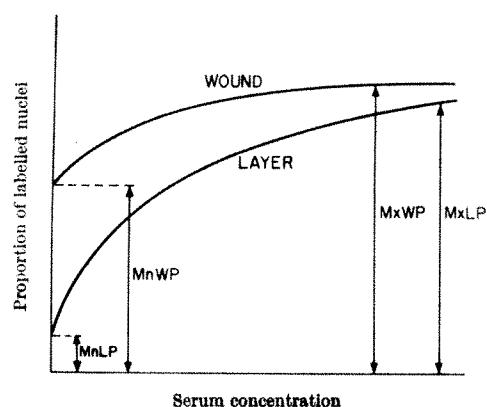


Fig. 5. Scheme indicating the quantities involved in the determination of the physiological parameters.

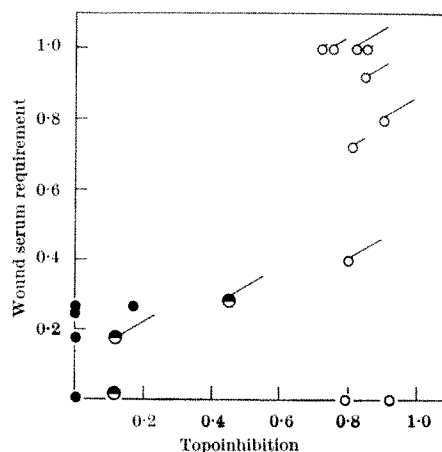


Fig. 6. Correlation diagrams for WSR, TI and CTI. The data of Table 3 are used. ○, Untransformed cells; ◐, transformed cells; ●, "flat" revertants of SV3T3 cells. The length of the line attached to some of the symbols is proportional to CTI.

of epithelial cells). Another is to prevent irreversible cell changes, ultimately leading to cell death; the last is concerned with mitosis.

Different factors in serum may be responsible for the different roles. For instance, the cell-survival factor may be different from that involved in WSR, as suggested by the results of Table 1, and also by results of others (personal communication from M. G. P. Stoker).

The control of multiplication of cells varies according to a combination of many physiological characteristics of the cells, of which five can be identified: WSR, TI, CTI, the serum requirement for survival, and for mitosis. For different cells, the quantitative value of each characteristic differs.

These factors play an important part in generating the morphology of cell cultures in standard conditions (that is, with 10 per cent serum in the medium). It is likely that the "thin" or "flat" fibroblastic cultures—that is, with low saturation density—such as 3T3 or BALB/c-3T3, derive their characteristics mostly from their requirement for large amounts of serum, especially to combat TI (CTI effect) and perhaps for mitosis. It is likely, furthermore, that these cultures exhaust serum at a high rate. High TI in itself does not determine a flat phenotype at high serum concentrations owing to the CTI effect of the serum. In fact, ME cultures, which have high TI but also high CTI (and probably also a low rate of serum exhaustion), are not "flat", and reach a rather high saturation density. BHK cells are situated in between, having a moderately high serum requirement and apparently a lower rate of exhaustion. The lines that are commonly used as "normal" in transformation experiments (3T3, BALB/c-3T3 and BHK) are therefore pseudonormal and differ considerably from truly normal cells (such as ME). These lines may have originated by several genetic changes, some in the direction of transformation, and others causing a high serum requirement for CTI and possibly also a high serum removal rate. This possibility is supported by the great ease with which 3T3 and BALB/c-3T3 cells give rise to spontaneously transformed derivatives, and by the mild oncogenicity of BHK cells in hamsters. The use of these pseudonormal cells as normal cells may lead to misinterpretations in many kinds of experiments. For instance, in the study of viral transformation, they may require fewer virus-induced changes than normal cells in order to become transformed.

Characterization of Transformed Cells

The transformed phenotype seems to result chiefly from minimal or absent TI and the concomitant absence

of serum requirement for CTI (Fig. 6). These cells also have low WSR, but in this respect they are no different from epithelial cells. Serum requirements of transformed cells for mitosis and for survival, however, are not small. These physiological properties are, to a large extent, independent of the morphological characteristics of the cultures, for several "flat" revertants (as described by Pollack⁹) would still be classified as transformed on the basis of WSR and TI, whereas others show intermediate properties (see Fig. 6). All the "flat" revertants, however, show a higher serum requirement for mitosis than SV3T3 cells. This shows that genetic changes can cause a dissociation of some physiological properties from others and from morphological properties. The latter point has already been demonstrated by other means (personal communication from M. G. P. Stoker). The physiological properties described in this article can contribute to a more precise definition of the transformed state.

Because transformed cells grow as neoplastic cells in animals, topoinhibition is probably an important mechanism regulating cell multiplication in organisms in normal conditions. The humoral factors that also control cell multiplication in animals may interact with topoinhibition. The observation that when cells are transformed by viruses they change simultaneously in several physio-

logical parameters (for example, TI and WSR) contrasts with the apparent independence of these parameters of each other. There is no contradiction, however, in these observations, for the transforming virus may affect several cellular functions through the simultaneous expression of several of its genes. Studies with viral and cellular mutants, now in progress, may clarify this point.

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Genetics of a Molluscan Vector of *Schistosomiasis*

by

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A complex of several genetic factors determines the susceptibility of juvenile *Biomphalaria glabrata* to infection by *Schistosoma mansoni*. A simple dominant factor can cause juvenile susceptible snails to become refractory to the parasite at maturity. Stocks of such adults may prove to be of use in genetic control of *S. mansoni* in endemic areas.

LITTLE is known about molluscan genetics; studies are urgently needed in particular on the role of genetics in the transmission of diseases by molluscs and in molluscan systematics. The significance of genetics of insect vectors of disease has been demonstrated^{1,2}. We now report studies on the genetics of *Biomphalaria glabrata* (= *Australorbis glabratus*), intermediate host and vector of *Schistosoma mansoni* and a unique subject for molluscan genetic studies.

As indicated by geographic distribution, habitat range, and morphology, *B. glabrata* has remarkable variability³. It has a diploid chromosome number of 36 (ref. 4). Its generation time may be as short as a month⁵, and a snail may continue reproduction for more than a year. These snails are hermaphrodite and they can reproduce by self-fertilization through many generations, facilitating genetic selection. When two snails are mated, however, cross-fertilization usually dominates^{6,7}. Because suppression of self-fertilization is not always complete, the expression "post-cross" is used to distinguish a snail's progeny after mating from those before mating. Pigment markers were used to identify cross-fertilized individuals among the "post-cross" progeny.

Pigment Markers

Albinism is monofactorial and is recessive to wild type pigmentation⁸, and it has been used as a genetic marker

in systematic and other studies^{6,7,9-11}. A cross between a Brazilian albino and a Puerto Rican wild type pigmented snail produced the albino and wild type stocks in my laboratory¹². A laboratory mutant of this albino stock, "blackeye"¹³, has pigmented eyes and may have some black mantle pigment, but it lacks the black body and collar pigment which are characteristic of the wild type. Blackeye (*c^b*) is a third allele which is dominant over the albino (*c*) but recessive to wild type (*C*). (These pigment types were formerly referred to as *B*, *A*, and *C* respectively.) Selfing of a heterozygote such as *Cc* or *c^bc* (Fig. 1) consistently results in a 3:1 ratio showing that albinism is recessive and that reproduction is by self-fertilization, not parthenogenesis. When two such heterozygotes are mated, reciprocal cross-fertilization results in the post-cross phenotypic ratio 2:1:1 (*C:c^b:c*) from both snails. All blackeye progeny of the *Cc* parent are known to be the result of cross-fertilization, and all wild type progeny of the *c^bc* parent.

By selection and selfing through several generations, wild type and blackeye clones with and without black mantle spotting patterns were derived, including heterozygotes carrying the recessive albino genes. Mantle spotting seems to be a multifactorial character, but serves as a marker for one generation, spotted (*S*) being dominant over unspotted (*U*) (my unpublished work). Five pigment phenotypes (*c*, *c^bU*, *c^bS*, *CU*, *CS*) are now

available. Although unable to produce black pigment, the albino carries and transmits the mantle pigment pattern. This produces a 3-way cross as shown in Fig. 1. An albino from an unspotted stock can be mated with the other four pigment types, either in series or concurrently, and the "male" parent of each of the albino's offspring can then be identified. While most experimental matings involve one pair of individuals, in this case information could be obtained on reproduction in a natural population where the individual has the opportunity for promiscuous mating with several associates.

Tentacle and Eye Variation

Tentacle abnormalities have been observed in various snail species¹⁴⁻¹⁶. In *B. glabrata*, the tentacles are sometimes double, branched, bulbous or missing on either or both sides; and double, displaced or missing eyes are also found (Figs. 2 to 5) (my unpublished work). Tentacle and eye variation shows inheritance patterns similar to the tumorous head character in *Drosophila melanogaster*^{17,18}.

Pulmonary Cavity Occlusions

A hereditary structural aberration, a pulmonary cavity occlusion consists of a tissue mass in the parietal wall of the pulmonary cavity (Fig. 7). Selection and selfing resulted in clones in which frequencies of pulmonary cavity occlusions increased to as much as 70 per cent of a snail's progeny. Mating snails from such clones with normal stock snails results in post-cross F_1 progeny of both parents developing pulmonary cavity growths. In many cases the growths completely occlude the pulmonary cavity, preventing retraction of the snail into its shell.

Sublethal Characters

Another hereditary aberration consists of a median head bulb (Figs. 5, 6). Selection and isolated self-fertilization have shown inheritance of this, and in clones showing the head bulb snails which appear normal also transmit the character to their progeny. It is associated with a sublethal condition—egg clutches show about 20 per cent surviving snails, the other 80 per cent dying either in early cleavage or after abnormal development. The highest frequency of surviving progeny with the median head bulb was 30 per cent.

Spire formation is also associated with a sublethal effect (my unpublished work) (Figs. 8, 9). Spired snails were occasionally seen in colonies of *B. glabrata*. When isolated, they either failed to reproduce or produced only normal progeny. By observing developmental stages in egg clutches, however, we selected for consistent occurrence at a low frequency (10 per cent) of spire embryos. The egg clutches usually contain less than 50 per cent surviving infants, the remainder (including most of the spire embryos) dying in early cleavage or following abnormal development (Fig. 9). When we mated snails from such clones with stocks in which neither the spired snails nor the sublethal effect had been observed, post-cross progeny of both parents showed the characters.

Pearl Formation

Formation of spherical pearls (Fig. 10) of calcium carbonate in the extra-pallial fluid in depressions in the mantle was first noted in a clone of albino *B. glabrata*. Most of the

pearls are eventually incorporated in the shell. Selfing and mating studies indicate that pearl formation (prl) is inherited as a simple recessive factor, with absence of pearls (Prl) dominant (my unpublished work).

Polyembryony

Polyembryony has been reported for many snail species and is not uncommon in *B. glabrata*. The relationship of the multiple embryos is unknown in most cases, but "dizygotic twins"¹⁹ and "monozygotic conjoined embryos"²⁰ have been reported. Frequencies of polyembryony throughout various clones of *B. glabrata* indicate this is an inherited tendency. Observations on pigment marked heterozygous snails suggest that most of the multiple embryos in *B. glabrata* involve multiple zygotes in the same capsule.

Haemolymph Pigment

Snail haemolymph has been used in systematic and other studies²¹⁻²⁶. Intraspecific genetic variations in the chemical constitution of haemolymph are pertinent to such studies, and may also affect host-parasite relations. A marked increase in haemoglobin with growth from juveniles to adults has been reported²¹. By selection and selfing, we derived clones of *B. glabrata* showing variation in degree of red haemolymph pigment and in time of expression of such variation—both juveniles and adults with red haemolymph, juveniles deficient but adults with red pigment, and both juveniles and adults deficient. In some clones, snails with completely colourless blood occur consistently but in low frequency, but they rarely survive.

Susceptibility to Infection

A genetic basis for variations in susceptibility of *B. glabrata* to infection with *S. mansoni* has been proposed²⁷. Most reported studies on susceptibility have dealt with adults from interbreeding snail populations, from either the field or laboratory colonies.

In our studies on juvenile susceptibility, snails 1-4 mm in diameter were individually exposed to five miracidia of a Puerto Rican strain of *S. mansoni*. Each snail was examined 7-10 days after exposure and those in which developing sporocysts were not evident were again exposed

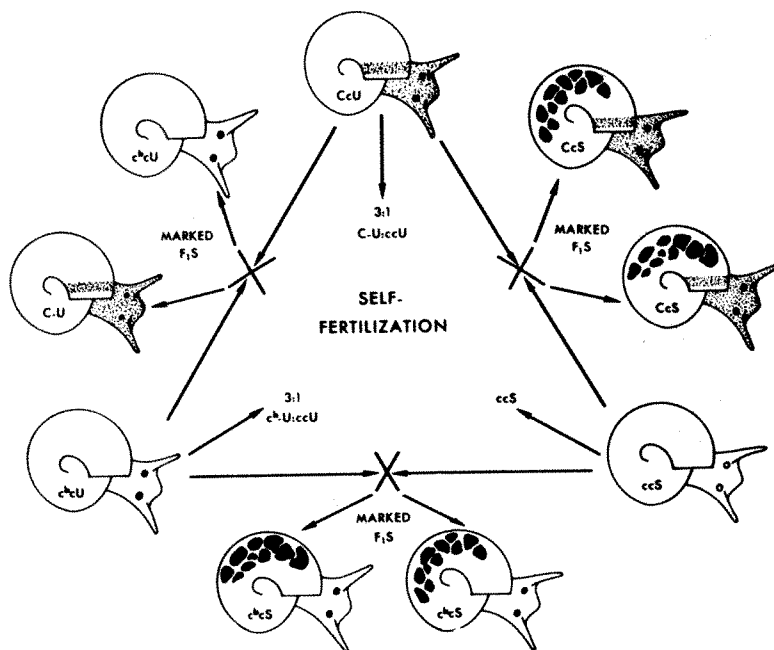


Fig. 1. Diagram illustrating: pigment markers in *B. glabrata*; wild type (C), blackeye (c^b), albino (c); spotted mantle (S), unspotted mantle (U); F₁ phenotypes qualitatively demonstrating cross-fertilization are depicted.

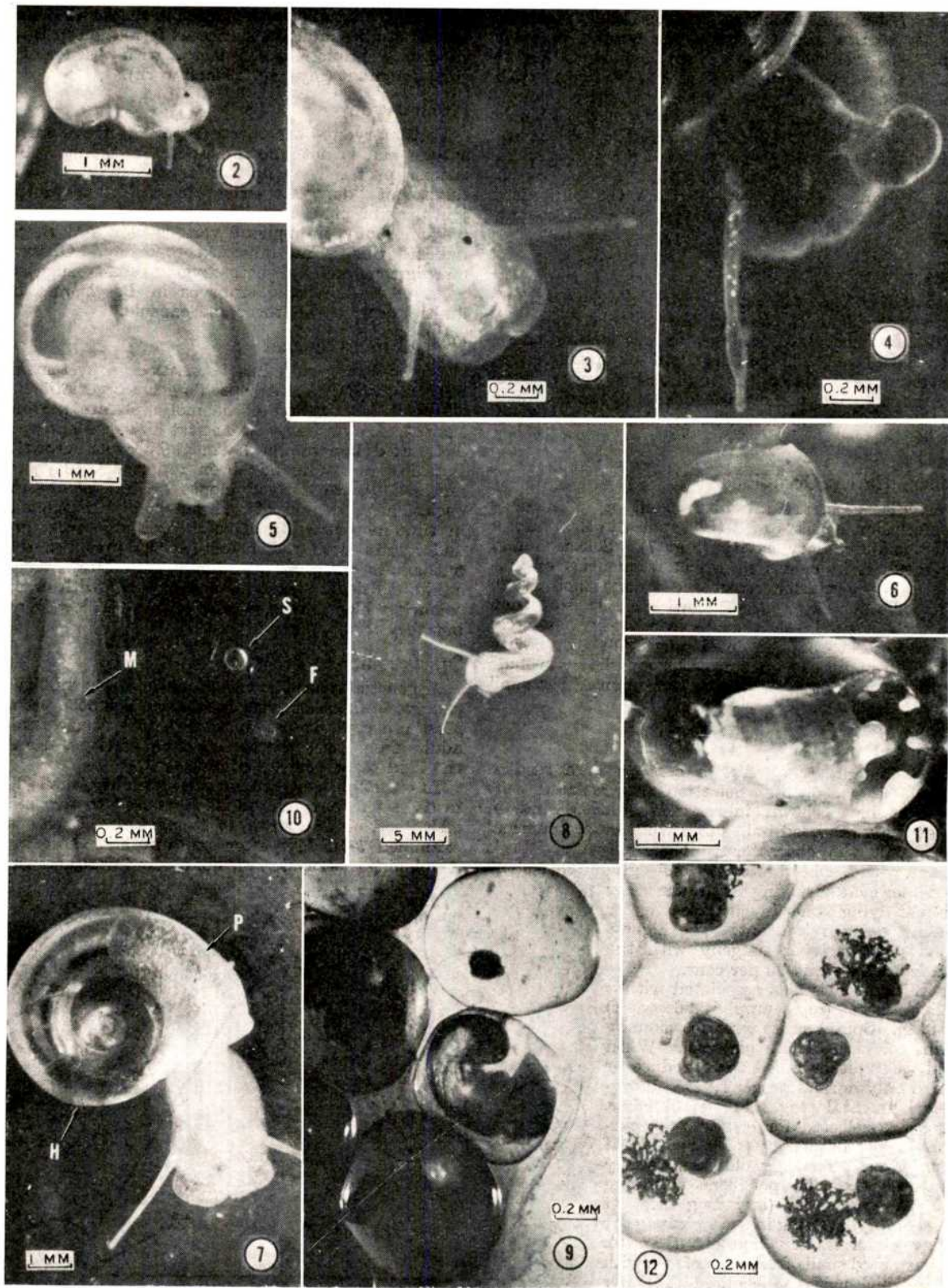


Fig. 2. Juvenile blackeye *B. glabrata* with double right tentacle and displaced right eye, $\times c. 10$.

Fig. 3. Head of same snail as Fig. 2, showing displaced right eye, $\times c. 21$.

Fig. 4. Snail with bulbous left tentacle, $\times c. 21$.

Fig. 5. Albino with bulbous right tentacle and median head bulb, $\times c. 10$.

Fig. 6. *B. glabrata* with median head bulb, $\times c. 10$.

Fig. 7. *B. glabrata* albino with pulmonary cavity occlusion (*P*); location of heart indicated (*H*), $\times c. 5$.

Fig. 8. *B. glabrata* with median head bulb, $\times c. 10$.

Fig. 9. Part of egg clutch of spire stock snail, showing developing spire embryo, two offspring dead in early cleavage, and one dead following abnormal development, $\times c. 21$.

Fig. 10. Part of albino *B. glabrata* showing mantle collar (*M*), pearl incorporated in shell (*S*), and free pearl in process of formation in depression in mantle (*F*), $\times c. 21$.

Fig. 11. Pattern of six apertural lamellae in young *B. glabrata* 4.0 mm in diameter.

Fig. 12. Part of egg clutch from hybrid snail showing two types of intracapsular inclusions; "dendritic" and "flakes", $\times c. 21$.

to five miracidia. Snails from a juvenile susceptible stock were used as controls with each exposure and re-exposure. Snails were checked for cercarial shedding at 6 weeks and at about 10 weeks. We considered infection as including any observed parasite development—primary sporocyst, secondary sporocyst or cercariae.

A cross was made first between a susceptible blackeye/albino heterozygote and a refractory homozygous wild type pigmented snail. Descendants of the susceptible parent by selfing produced progenies which were 90–100 per cent experimentally infected; post-cross F_1 s were 45 per cent infected. Descendants of the refractory parent by selfing produced progenies with 0 per cent infected; post-cross F_1 s 45 per cent infected. Several reported crosses between populations of “high” and “low” susceptibility have shown intermediate susceptibilities^{12, 28, 29}.

We isolated and selfed a series of forty F_1 s, twenty from each parent. Twenty-five of these F_1 s survived and produced progenies showing juvenile susceptibilities ranging from 0 per cent to 75 per cent. Stocks showing 100 per cent juvenile susceptibility and stocks showing no susceptibility were derived by selection and selfing through three generations. Genetic differences are not revealed so long as several susceptible clonal stocks are maintained by selfing, and tests show 100 per cent juvenile susceptibility. Tests on refractory clonal stocks showing no susceptibility also fail to demonstrate genetic variation. After selfing, a refractory wild type snail was mated as a male with ten albino or blackeye snails in series. Differences in susceptibility in cross-fertilized F_1 s of several susceptible albino mates suggested that these susceptible snails probably represented at least three different genotypes. When a blackeye refractory stock snail was used as a male in crosses with the same series of susceptible albinos, the susceptibility of cross-fertilized F_1 s differed from that of the refractory wild type parent, thus suggesting that the refractory stocks differed genetically. In matings between refractory snails from different stocks, some susceptible cross-fertilized F_1 s resulted, showing that refractory snails can carry susceptibility genes. From the many crosses conducted, I conclude that juvenile susceptibility is determined by a complex of several genetic factors.

In *B. glabrata* some snails susceptible as juveniles become refractory as adults^{21, 27}. Similar age effects have been reported for *Bulinus*³⁰ and lymnaeid snails³¹. The opposite effect, with susceptibility increasing with age, has been reported for some other planorbisid snails³². We exposed twenty-two adult snails descended from the susceptible refractory cross to twenty-five miracidia per snail. All proved to be refractory. We then isolated ten offspring from each of the snails derived from the above cross whose progenies showed high juvenile susceptibility (85 per cent–100 per cent). At the onset of egg-laying, thirty-one survivors were each exposed to twenty-five miracidia. All proved to be adult refractory. Control adults from the laboratory population became infected after exposure. Thirty-four offspring obtained by selfing from a juvenile susceptible stock snail were then exposed at various sizes. Thirteen offspring, less than 4 mm in diameter, were each exposed to five miracidia per snail. All became infected. Each of the remaining twenty-one offspring which had been reared in isolation was exposed to twenty-five miracidia after the first three had laid eggs at 10 mm diameter. One snail at 5 mm and two at 6 mm became infected, but none of the seven at 7 mm, four at 8 mm, four at 9 mm, or three at 10 mm became infected. These results suggest that “adult insusceptibility” in juvenile susceptible snails is initiated by developmental changes associated with maturation just before the onset of egg-laying.

Clonal stocks from two sibling albinos were followed through three generations by isolations and selfing. One

stock included both refractory and susceptible adults with the refractory snails predominating and with frequencies suggesting the presence of a single factor with susceptibility recessive. The other stock yielded 72/75 exposed adults susceptible, suggesting that these were homozygous recessive. In three crosses between juvenile susceptible/adult susceptible and juvenile susceptible/adult refractory snails, F_1 s produced 90–100 per cent juvenile susceptible, 0 (0/22) adult susceptible. F_2 progenies of seven isolated hybrid F_1 s showed 20 per cent (9/45) adult susceptible. These F_1 s were backcrossed to adult susceptible snails resulting in 52 per cent (25/48) backcross progeny which were adult susceptible. Additional crosses and isolations support the occurrence of a genetic factor with adult insusceptibility dominant and susceptibility recessive.

We now have three kinds of snail stocks with respect to developmental susceptibility to Puerto Rican *S. mansoni*: susceptible at any age, juvenile susceptible but adult refractory, and refractory at any age. On the basis of these preliminary results I postulate that a complex of several genetic factors determines juvenile (basic) susceptibility, insusceptibility being related to a deficiency of some requirement for parasite development; and that a simple, dominant factor (Mr) can cause juvenile susceptible snails to become refractory at maturity. There may be additional factors which modify the influence of this maturity factor. Implications of this inherited developmental variable in evaluating susceptibility either in the field or laboratory are obvious. In the epidemiology of schistosomiasis, only adult snails are usually considered but, as shown here, juvenile snails may be very significant. Infected juvenile *B. glabrata* have shed cercariae while only 1–8 mm in diameter.

Geographic variations involve the genetics of both host snail and trematode parasite^{27, 28, 33–35}. Preliminary observations have been made on host-parasite relations with five geographic strains of *S. mansoni* and eight different populations of *B. glabrata*. The *S. mansoni* strains which were tested fall into three groups on the basis of their infectivity; the snails into four groups on the basis of susceptibility. The results suggest that these differences in susceptibility of the snail may be related to variations in the requirements of the strains of the parasite.

Aestivation

Another physiological character is combined with morphological and behavioural features. Paraense described the occurrence of “apertural lamellae” (Fig. 11) and associated diapause in some small *B. glabrata* in Brazil³⁶ which had a tendency to climb spontaneously out of water and aestivate. I reported similar observations for *B. glabrata* in Puerto Rico³⁷. Physiological changes precede diapause, adapting these snails for prolonged aestivation³⁸. Lamella production and capacity for aestivation are inherited and genetically transmitted in cross-fertilization³⁹. Spontaneous aestivation provides insurance against unpredictable drought or chemical molluscicides⁴⁰. The efficiency of molluscicides would be affected by inheritance of avoidance behaviour or resistance⁴¹. A mutation in an aestivating *B. glabrata* has an excellent chance of survival, because at the end of the dry period the new population is started by only a few surviving snails⁴², and in some cases one isolated snail might repopulate a local habitat by self-fertilization.

Other Inherited Characters

The variations and aberrations described all occurred in a stock which has been under laboratory colonization for more than 15 years. We observed additional characters, the frequencies of which suggest inheritance: (1) Shell: colour amber or clear, high or low (umbilicus deep or shallow), multiplanar whorls (contorted shell), body whorl expanded. (2) Pigmentation: white pigment

granules of head and tentacles numerous or scarce; shape, size and distribution of mantle spots; kidney of newly hatched snails straw-coloured, magenta-coloured or blue to colourless; eyes of newly hatched snails red or black. (3) Egg clutches: contents of egg capsule clear, cloudy, with needle or flake-like inclusions, or with dendritic inclusions (Fig. 12); size, number and arrangement of egg capsules. (4) Growth: diameter at onset of egg-laying.

Genetic Control

Stocks of *B. glabrata* refractory to *S. mansoni* may prove of use in genetic control of this parasite, but it is essential to clarify the genetics of the factors involved and develop stocks carrying refractory characters only. Introduced into an endemic area, such stocks might be expected to hybridize with the local susceptible population resulting in a range of susceptibility combinations in succeeding generations, but transmission should be reduced. The presence of refractory *B. glabrata* in field populations should also have an effect on the miracidia⁴³ resulting in reduced infection frequency in the susceptible snails. If snails selected for a combination of insusceptibility and aestivation adaptation^{38,39,44} were introduced, a more rapid shift toward insusceptibility might be achieved following population reduction by drought or mollusciciding. Introduction of refractory snails would avoid the unpredictable hazard to the "balance of nature" of introducing new species (competitors, predators or parasites) in biological control.

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Lysolecithin and Cell Fusion

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Lysolecithin will induce cell fusion in several different types of cell, and both multinucleated syncytia and heterokaryons can be formed.

INTEREST in the fusion of biological membranes has recently been stimulated by investigations on the biochemistry of secretion¹, on the roles of membranes in the lysosomal vacuolar system² and, in particular, by work on the fusion of cells that is induced by viruses^{3,4}. For cell fusion induced by inactivated Sendai virus, it has been suggested that the virus provokes a general change in the plasma membrane of cells, which induces fusion as a secondary effect that no longer requires the presence of a virus⁵. More recently it has been shown, however, that the plasma membranes of Ehrlich ascites cells become degraded in contact with virus particles, and that this leads to the communication of the cytoplasm of adjacent cells through the breaks⁶. Other investigators have postulated that the Sendai virus contains a "cell wall destroying enzyme"⁷. Because phospholipase A com-

pletely abolishes the ability of Newcastle disease virus to cause cell fusion, it has been suggested that the phospholipids of the viral membrane are involved in the fusion process⁸. It has also been proposed that cellular lysosomal enzymes, rather than viral enzymes, are concerned in virus-induced cell fusion^{9,10}.

We now report experiments designed to test a working hypothesis for the fusion of biological membranes which is discussed in the following article¹¹. On this hypothesis, membrane fusion would be facilitated by factors, such as lysolecithin, that would be expected to favour a micellar organization for the lipids of biological membranes^{12,13}, rather than a bimolecular leaflet structure. We have therefore investigated whether lysolecithin can induce erythrocytes to fuse and form homokaryons: a brief report of cell fusion observed with hen erythrocytes has been published¹⁴. We have also studied the effects of

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lysolecithin on other blood cells and on LS fibroblasts. Several different cell types have been induced to fuse, and the formation of heterokaryons by inter-species fusion has been observed.

The lysolecithin used in the initial experiments was given by Dr G. R. Webster. It was prepared from ovoidlecithin with snake venom (*Agkistrodon p. piscivorus*), and was chromatographically pure (fatty acid composition: 67.2 per cent palmitic, 32.8 per cent stearic acid). A comparable preparation, supplied by Lipid Products (Epsom), gave similar results. Solutions of lysolecithin in 150 mM NaCl solution were prepared immediately before use. In some experiments siliconized glassware was used in the preparation of the solutions of lysolecithin: this procedure had no observable effect on fusion. The fibroblasts of the mouse strain LS cells, originally isolated by Professor J. Paul from the L (clone 929) strain, were derived from a culture provided by Professor S. J. Pirt. Bright-field and phase-contrast light microscopy were undertaken with a Vickers M26 inverted microscope, and electron microscopy was carried out with an AEI EM6b electron microscope.

Polysyncytium Formation

Hen erythrocytes, which were isolated and prepared for experimental work as described previously¹⁴, were rapidly lysed on treatment with lysolecithin. Light microscopy revealed that, before fusion, the ghosts tended to adhere to one another, sometimes in very large groups or chains. This observation is of considerable interest in relation to the factors governing cell adhesion¹⁵, and it indicates that one way in which adhesive cells may differ from non-adhesive cells could be in their relative content of membrane lysolecithin. Studies with the electron microscope showed that in some experiments many groups of cells had fused to form syncytia within 30 s of contact with lysolecithin. Part of such a syncytium is shown in Fig. 1 (page 813). A more magnified view of the apex of a large syncytium, where it is only one nucleus wide, clearly demonstrates cell fusion (Fig. 2). Neither fusion nor lysis occurred when erythrocytes were incubated for short periods at 37° C in buffer at pH 5.7 in the absence of lysolecithin. Some evidence of fusion was obtained with lysolecithin at pH 7, but a low pH was used in the experiments described here in an attempt to reduce electrostatic repulsion between cells. It may be relevant that the formation of giant cells in tissue cultures of chicken macrophages occurs most readily between pH 5.5 and 6.0 (ref. 16).

Membranes which were induced to fuse by treatment with lysolecithin were at the same time highly unstable. This instability frequently resulted in a complete breakdown of the multinucleate cells. In the experiment from which the electron micrographs of Figs. 1 and 2 were taken the syncytia, which were present after 30 s, rapidly disintegrated and were virtually absent in specimens fixed 3 min after the addition of lysolecithin. It may be significant that membrane disintegration, occurring 6–8 h after cell fusion, has been observed with syncytia formed both from sheep choroid plexus cells and from baby hamster kidney cells treated with visna virus particles¹⁷.

The precise sequence of morphological changes which occurs during fusion is of interest and preliminary findings suggest that the process may involve the formation of cytoplasmic bridges, as with cell fusion that is initiated by inactivated Sendai virus⁵. Other phenomena that we have observed with lysolecithin-treated erythrocytes include a loss of membrane rigidity, the formation of long, winding membranous processes derived from the plasma membrane, and the inclusion of closely packed membranes within the multinucleated syncytia. From observations on virus-induced cell fusion it has been concluded that, although the main mechanism of nuclear fusion involves post-mitotic reconstitution of several nuclei as a single large nucleus, interphase nuclei showing

varying degrees of fusion could also be seen in the virus-induced heterokaryons⁴. Fused nuclei were recognized in experiments with inactivated virus by their size, irregular shape and abnormally large number of nucleoli. In these studies with erythrocytes, nuclei of bizarre shape, but near normal size, were observed which may have been formed as a result of nuclear fusion following cell fusion. Ultrastructural work on the effects of lysolecithin on red cell morphology during cell fusion is being continued, and will be published elsewhere.

Avian erythrocytes are useful for studies on cell fusion. They are readily obtainable and, being nucleated, they make fusion easy to detect. In addition, because mature erythrocytes do not divide, there can be no confusion between fusing cells and dividing cells. Erythrocytes are nevertheless highly specialized, and it seemed possible that lysolecithin might not be able to induce the fusion of other cell types. That lysolecithin-induced fusion is not restricted to erythrocytes was initially shown, however, by the finding that heterophils are occasionally incorporated within the multinucleated syncytia that are formed when a preparation of erythrocytes contaminated with white blood cells is treated with lysolecithin¹⁴. Evidence that lysolecithin is able to cause fusion of various cell types has been provided by the following investigations.

Experiments were performed with exponentially grown mouse fibroblasts of the LS strain (grown in Eagle's minimum essential medium containing 10 per cent foetal calf serum)—these cells normally show relatively little adhesion to one another. Initially, treatment of the cells with lysolecithin (330 µg/ml.) in Hanks balanced saline solution led, even after only 30 s at 37° C, to membrane disruption in many of these cells. Attempts were then made to induce fusion of LS fibroblasts with one another by using sub-lytic quantities of lysolecithin. Occasionally clumps of apparently fused cells were seen with the light microscope, but these experiments met with little success: it seems that the membranes of the LS cells were more susceptible to lysis than to fusion under the conditions used.

Heterokaryons

In experiments on the formation of heterokaryons from LS fibroblasts and hen erythrocytes, the exponentially grown fibroblasts were washed twice in Hanks balanced saline solution, and resuspended in 150 mM NaCl just before use. Red cells were prepared as before¹⁴, and also resuspended in 150 mM NaCl solution before use. The two cell populations were mixed and added to an equal volume of 150 mM sodium acetate buffer (pH 5.6). Lysolecithin (dissolved in saline solution) was then immediately added. This treatment led to clumping and cell adhesion, observable by light microscopy. After 30 s at 37° C with lysolecithin, cell fusion had occurred and heterokaryons, as well as multinucleated erythrocytes, were seen with the electron microscope. These phenomena were not observed in control experiments without lysolecithin, although there was some evidence of cell damage that may have resulted from the low pH. Fig. 3 is a thin section of a portion of a multinucleated heterokaryon in which a fibroblast nucleus is closely associated with an erythrocyte nucleus. No plasma membranes can be distinguished between the nucleus of the fibroblast and the adjacent erythrocyte nucleus.

Another interesting example of heterokaryon production was observed when a preparation of hen mixed blood cells and LS fibroblasts was treated with lysolecithin. Fig. 4 shows part of a single LS fibroblast with which thrombocytes¹⁸ have fused at a number of different locations. The differing appearances of the cytoplasm of the two cell types can be seen better in Fig. 5, which also clearly demonstrates the absence of plasma membranes between the fibroblast and one of the thrombocytes. In addition to the heterokaryons illustrated here, hetero-

karyon formation has been observed with hen erythrocytes and mouse macrophages. The morphology of these heterokaryons resembles that of the hybrids formed from hen erythrocytes and LS fibroblasts.

In preliminary experiments on the stability of multinucleated cells produced by lysolecithin-induced fusion it has been found possible, by adding de-fatted serum albumin to the lysolecithin-treated cells, to arrest the associated degradative action of lysolecithin on cell membranes. Multinucleated cells can be obtained in this way which are apparently stable for several hours after the fusion process, as judged by light microscopy.

With regard to the proportion of cells undergoing fusion, it seems that this depends both on the concentration of lysolecithin and on the concentration of cells. Fusion was commonly seen in many experiments: the greatest incidence of fusion observed so far was in the experiment of Fig. 3, where electron microscopy indicated that about 20 per cent of the cells were involved in fusion.

Implications

These seem to be the first studies in which cell fusion has been induced, leading to the production of both multinucleated syncytia and heterokaryons, by treating cells with a simple chemical substance that is known to have a direct effect on the integrity of lipoprotein membranes. Fischer and his colleagues¹⁹ have previously noted, without comment, that fusion of cellular projections occurs in peritoneal macrophages treated with lysolecithin. Their light micrographs do not, however, enable a clear distinction to be made between fusion and adhesion.

The fusion of cell membranes caused by lysolecithin is consistent with the suggestion²⁰ that the ability of primary lysosomes to fuse with digestive vacuoles, and with the plasma membrane, may be due to lysosomal membranes containing a relatively high proportion of lysolecithin that results from the activities of lysosomal phospholipase A₁ or A₂ (refs. 21-23). Our findings also lend indirect support to the idea that lysolecithin may be concerned in the secretion of catecholamines from both normal and tumour chromaffin cells^{24,25}. In relation to secretions from tumour cells and the possibility that the invasive properties of malignant cells may involve extracellular release of lysosomal enzymes²⁶, it is interesting that some tumours contain a relatively high proportion of lysolecithin²⁷. The present observations also favour earlier suggestions that lysolecithin may participate in the release of histamine from mast cells²⁸ and in phagocytosis¹⁹. In addition, because there is circumstantial evidence that lysolecithin may be involved in demyelination²⁹, it may be pertinent to note that both demyelination and cell fusion have been reported with visna virus, canine distemper virus and mouse hepatitis virus¹⁷. It seems, however, that lysolecithin apparently does not participate in fusion of kidney cells by a para-influenza virus³⁰.

It could be argued that the lytic properties of lysolecithin, and the instability of lysolecithin-treated membranes preclude the idea of lysolecithin participating in membrane fusion occurring in living cells. We envisage, however, that in the latter situation lysolecithin may be produced at highly localized sites so that the overall integrity of the membranes concerned are not threatened *in vivo*, as they are when cells are treated with exogenous lysolecithin. It also seems likely that any cell fusion induced by lysolecithin *in vivo* may involve transient changes in the level of lysolecithin, and it may be significant that some cells can remove lysolecithin by two different reactions³¹, as well as producing it with phospholipase A. Together, the degradative and regenerating reactions provide a potential control system whereby the structures of membranes may be changed to allow fusion to occur as necessary. It has, indeed, been suggested previously that lysolecithin might be a precursor of lecithin in the pinching

off of plasma membranes and in the fusion of phagocytic vacuoles with the lysosomes of granulocytes³².

One important possibility is that heterokaryon production by means of lysolecithin treatment might provide a more controllable method for the formation of hybrid cells than the treatment of cells with viruses currently being used in studies on genetics, differentiation and cancer research. The technical manipulations involved in virus-induced fusion could be avoided in this way. Also there would be no possibility, with hybrids produced by lysolecithin treatment, of attributing the behaviour of the cells to the effects of viral components. Furthermore, should cell hybrids become of value in the treatment of human cancer by immunological techniques³³, hybrids prepared without the use of viruses would be of considerable clinical importance. Preliminary experiments in this laboratory on the viability of cells fused with lysolecithin indicate that the new technique may be adapted routinely for the production of hybrid cells.

The ability of lysolecithin to induce fusion in a number of different cell types leads us to consider its implication in the molecular mechanisms that are involved in other instances of membrane fusion. Because lysolecithin-induced fusion is predictable from the hypothesis discussed in the following article¹¹, our observations seem also to support the possibility that the formation of micelles of lipid or lipoprotein within membranes, under the influence of a variety of appropriate agents, may be a general mechanism for the fusion of biological membranes.

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³³ Watkins, J. F., and Chen, L., *Nature*, **223**, 1018 (1969).

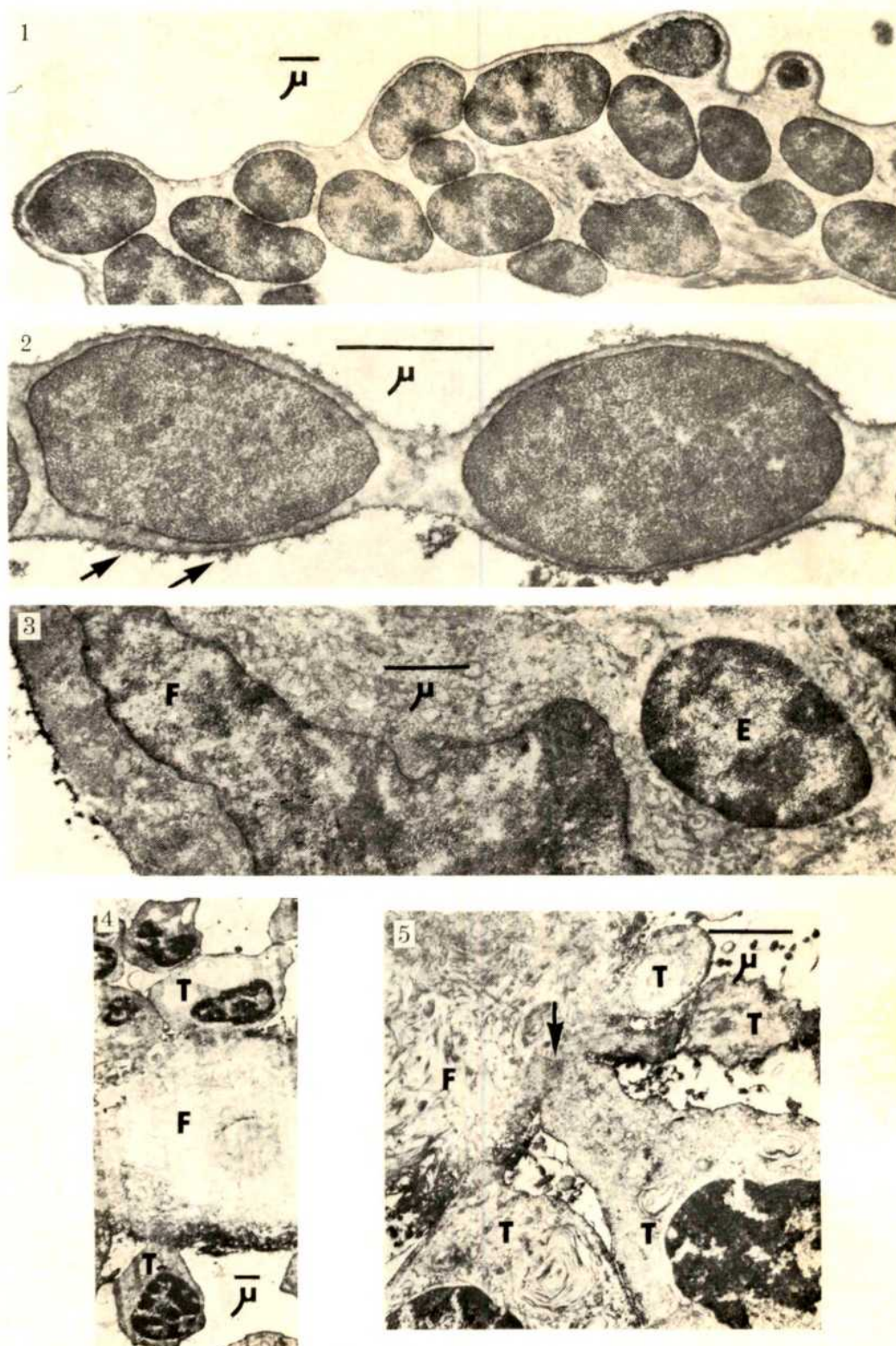


Fig. 1. Electron micrograph of a section through part of a large syncytium formed from hen erythrocytes in which nineteen nuclei can be distinguished. Erythrocytes were prepared and treated with lysolecithin as previously described¹⁴. The incubation mixture (1.7 ml.) contained 4×10^8 hen blood cells and 560 μg of lysolecithin in 106 mM NaCl and 44 mM sodium acetate buffer: final pH 5.7. After 30 s at 37° C with lysolecithin, the cells were fixed by the addition of ice-cold glutaraldehyde and they were then prepared for electron microscopy as before¹⁴.

Fig. 2. A section of part of a syncytium in which hen erythrocytes have fused lengthwise to form a chain of nuclei enclosed within a single plasma membrane. The outer surface of the membrane, stained by ruthenium red, is clearly observable (arrows). Reproduced with permission from ref. 14.

Fig. 3. An electron micrograph of part of a heterokaryon containing the nucleus of a hen erythrocyte (E) and the nucleus of a mouse LS fibroblast (F). The incubation mixture (1.3 ml.) contained 4×10^8 hen blood cells, 2.8×10^6 mouse LS fibroblasts and 240 μg lysolecithin in 92 mM NaCl and 58 mM sodium acetate buffer: final pH 5.7. Cells were fixed with ice-cold glutaraldehyde after 30 s at 37° C with lysolecithin.

Fig. 4. A low magnification electron micrograph of a mouse LS fibroblast (F) that has fused with a number of surrounding hen blood thrombocytes (T). The incubation mixture (1.7 ml.) contained 2.6×10^8 hen blood cells, 2.5×10^6 mouse LS fibroblasts and 560 μg lysolecithin in 106 mM NaCl and 44 mM sodium acetate buffer: final pH 5.7. The preparation was fixed with glutaraldehyde as in previous experiments.

Fig. 5. A detail of the syncytium of Fig. 4, showing region of fusion between a thrombocyte (T) and fibroblast (F) at higher magnification (arrow).

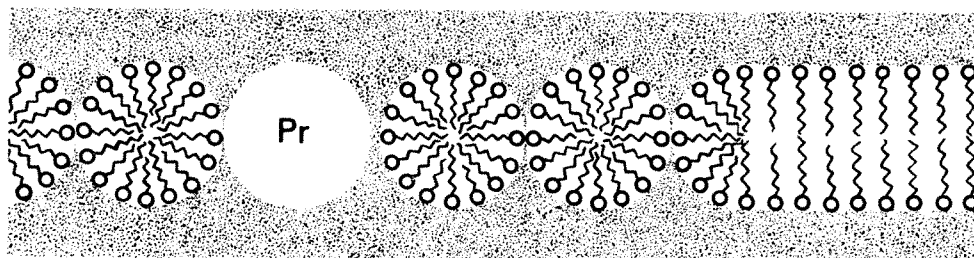


Fig. 1. Cross-sectional view of a membrane in which globular micelles of lipid are in dynamic equilibrium with a bimolecular leaflet of lipid⁴. A layer of protein and/or glycoprotein is shown on each side of the lipid layer. The structure of each lipid molecule is illustrated in diagrammatic fashion: only a polar head group and a non-polar moiety are shown, and the lipid may be phospholipid or non-phospholipid. One globular micelle of lipid has been replaced by a globular protein molecule (*Pr*) which may be a functional enzyme. (Reproduced from the *British Medical Bulletin*⁵ with the permission of the British Council.)

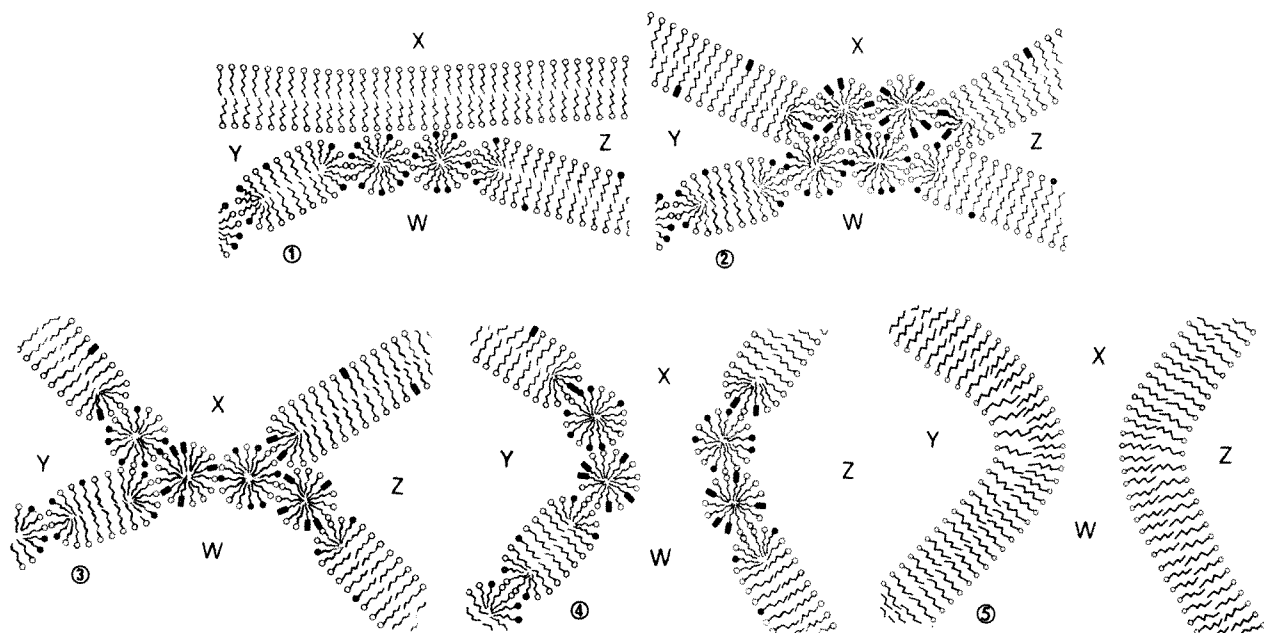


Fig. 2. This is intended to illustrate diagrammatically the possible involvement of micellar configurations of lipid molecules in the fusion of membranes. Stage 1: diagrammatic illustration of a cross-sectional view of the lipid molecules of parts of two adjacent membranes. In the upper membrane, the lipids are wholly in the form of a bimolecular leaflet. The lipids of the lower membrane are organized partly in a bimolecular leaflet and partly in globular micelles: specific, endogenous molecules, which may be either lipid (represented by ● with tail) or protein, are responsible for the stability of the globular micelles in the lower of the two membranes. It is postulated that fusion between two membranes will not occur if either (or both) of two adjacent membranes has its lipid molecules predominantly in the form of a bimolecular leaflet. Stage 2: as a result of the presence of an exogenous perturbing molecule, which may be either lipid (illustrated by ■ with a tail) or protein, a transition has occurred in the organization of the molecules of the upper of the two membranes. This membrane now resembles the lower membrane (as shown here) in having localized areas in which its lipids are organized in globular micelles. Stage 3: interdigitation of the globular micelles of the two adjacent membranes leads to a local fusion of the membranes into a single entity. Stage 4: breakdown of the unstable coalesced membrane-junction may simultaneously create and eliminate membrane-bound compartments. Stage 5: a final stage in membrane fusion may be the reversion of one or both membranes (as shown here) to the bimolecular leaflet form as a consequence of metabolic transformations involving one or more of the chemical components of the membranes. (In the context of pinocytosis, both the upper and lower membranes of stage 2 are considered to be part of the same plasma membrane. The molecules represented by ● and ■ with tails are to be regarded as identical in pinocytosis and also in cell fusion.) Stages 1-4 are reproduced from ref. 15 with permission of the North-Holland Publishing Co.

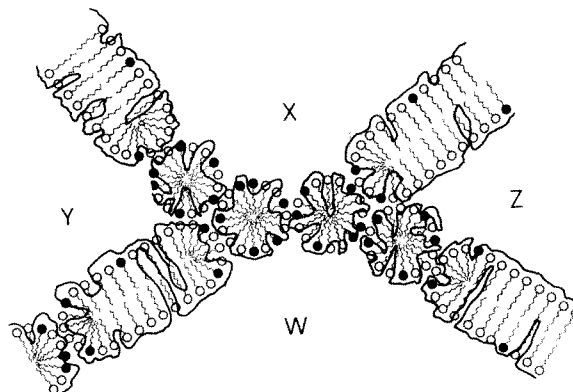


Fig. 3. A diagrammatic illustration (stage 3) of one way in which lysolecithin (● with tail) may interact with lipoprotein membranes, and cause membrane fusion by inducing changes in the organization of both the lipid and protein moieties of the two membranes. The presence of the lysolecithin may lead to the formation of globular micelles of lipoprotein which facilitate fusion. This figure was drawn by Mr R. A. Parker.

The Fusion of Biological Membranes

by

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A working hypothesis for the fusion of membranes is discussed. It is suggested that the process may involve phase changes in the lipids of membranes with the formation of micellar units of lipid or lipoprotein.

ALTHOUGH much is known of the structure and function of individual biological membranes, knowledge of the processes concerned in the fusion of membranes, a phenomenon so important in biology and medicine¹, is limited. Fusion in the lysosomal vacuolar system has recently been discussed in terms of "exoplasmic" and "endoplasmic" space by De Duve² and by Dingle³, who has suggested that membrane-bound vesicles will fuse only at a high value of surface tension. I now wish to draw attention to relationships between the structures of membranes and some of the factors that induce membrane fusion, and discuss relevant aspects of examples of membrane fusion. A possible molecular mechanism for the fusion of membranes is put forward on the basis of an earlier suggestion that the lipids of membranes may be arranged in globular micelles in appropriate circumstances instead of in a bimolecular leaflet^{4,5} (Fig. 1). Observations on the fusion of cells treated with lysolecithin, which are thought to be consistent with this mechanism, are reported in the preceding article⁶.

Phase Changes within Membranes

Okada *et al.*⁷ have proposed that cell fusion by Sendai virus involves first a virus-induced disconnection of the original plasma membranes, and then the connexion of membranes between adjacent cells. Any mechanism for membrane fusion which involves the physical removal or destruction of parts of adjacent membranes, however, seems unable to account for the fact that simple leakage of proteins, enzymes and subcellular organelles does not normally accompany membrane fusion. Thus disconnection of membranes is unlikely to be involved in the initial stages of the fusion reaction. Rather it seems more probable that a change occurs in structural organization which allows the altered membranes to participate in the fusion process and also to retain some degree of structural integrity.

Although membrane proteins are without doubt of great structural and functional importance, phospholipid molecules seem, nevertheless, to be a *sine qua non* for the formation of membranes in biological systems. I therefore suggest that the configuration of the lipid molecules of membranes is of primary importance in the process of membrane fusion, and that fusion will occur only if the lipid molecules are appropriately orientated. It is also proposed that the reorganization of membrane structure which occurs during fusion involves a phase-change in membrane lipids. This does not imply that the conformation of membrane proteins has no influence on the orientation of the lipid molecules. Indeed, membrane proteins and glycoproteins are probably principal factors in determining whether or not the lipids can adopt a configuration that will allow membrane fusion to occur. The stability and properties of bimolecular

lipid leaflets in aqueous solution have been examined theoretically by Haydon and Taylor⁸. They concluded that transitions from a bimolecular leaflet to aggregates of radially orientated molecules will occur if phospholipid molecules having a net charge, or wedge-shaped phospholipids, or non-phospholipid molecules, are introduced into the lipid leaflet. When dispersions of lecithin and cholesterol in water were investigated by the negative staining technique of electron microscopy, both spherulites and tubular structures were observed⁹, each tube apparently being made up of five parallel rows of globular lipid micelles having measurable (hydrophobic) diameters of about 40 Å. Although specific factors, such as the negative stain, may conceivably be responsible for the presence of globular micelles of phospholipid in such dispersions¹⁰, it is quite possible that conditions leading to the formation and stabilization of globular micelles may occur within biological membranes *in vivo*. The influence of various biochemical and physiological factors on this equilibrium, and the possibility that the proportion of micellar configuration might normally be very low, were stressed when the existence of globular micelles in membranes was initially considered⁴. Recently Danielli¹¹ has also commented that the important question is not whether the membrane has a specific structure (for example, bilayer or micelle), but rather how much of the membrane is present at one time in a particular structure or conformation, and how changes in these ratios are controlled by the cell. Stein¹² has suggested that the globular micelle may be the most stable arrangement for lipid molecules at the front of an advancing myelin figure, in view of the known change in birefringence which occurs at the ends of myelin forms. Observations made by the technique of freeze-etching led Branton¹³ to propose that most biological membranes are organized in part as bilayers of lipid and in part as globular subunits.

Globular Lipid Micelles and Membrane Fusion

Thermodynamic considerations indicate that the bimolecular leaflet is probably the natural unit of structure for lipid membranes^{8,14}, and the stability and lack of coalescence that biological membranes normally exhibit could well be a reflexion of the importance of the bilayer structure in these membranes. Membrane fusion may not occur if the lipids of one (or both) of the membranes concerned are almost entirely in the bimolecular leaflet configuration. Conversely, it is suggested that the primary requirement for the fusion of two membranes is that both of the membranes have a relatively high proportion of their phospholipid molecules in the micellar configuration. Any situation which favours the micellar organization rather than the bilayer should therefore tend to facilitate membrane fusion, although changes in the bilayer-micellar equilibrium may, of course, be second-

ary to a primary change that does not affect membrane lipids directly.

This hypothesis is illustrated diagrammatically in Fig. 2 (page 814) for the lipid molecules of two membranes which, in stage 1, show no tendency to fuse (Fig. 2:1). One of these membranes may be regarded as a plasma membrane that is relatively free from micelles. The other may be considered as part of a cytoplasmic organelle, for example, a primary lysosome which may normally have a relatively high proportion of micelles in its membrane¹⁵. Stage 2: any agent that increases the proportion of micelles in the plasma membrane (Fig. 2:2) might facilitate membrane fusion, for, if two apposed membranes both contain micelles, fusion may occur by interdigitation of the micelles as illustrated in Fig. 2:3. The arrangement depicted in Fig. 2:3, stage 3, could be described as a coalesced state of membranes. But whereas thermal motion and cytoplasmic streaming may allow the temporary formation of such a coalesced structure, these same factors will limit the lifetime of this complex junction of membranes. On breakdown of the junction, the membranes may either revert to the initial structures from which the junction was formed, or they may break up in the way illustrated in Fig. 2:4. In the latter instance, stage 4, area *W* (representing, for example, the interior of a secretory organelle) is brought into direct contact with area *X* (representing the exterior of the cell).

A final stage in membrane fusion may be the reversion of the structures of the membranes concerned to the bimolecular leaflet configuration. In some instances, reversion might not occur. In others it may be slow—old endocytotic vacuoles fuse less readily than newly formed vacuoles³, and a slow change in the structure of vacuole membranes may occur as the organelles move through the cytoplasm. By contrast, a relatively rapid reversion to the bimolecular leaflet structure probably follows the fusion of cells induced by viruses. Such a change may be necessary in order to confer stability on the resulting multinucleate cells. It is envisaged that the effects of the perturbing molecules which initiate the fusion process may be neutralized in stage 5 as a result of metabolic reactions, thus allowing the membrane lipids to revert to the more stable bimolecular leaflet configuration. This could, presumably, be achieved in many ways, depending on the perturbing molecule or molecules involved. For example, with cell fusion induced by lysolecithin⁶, the effective removal of lysolecithin by its conversion to lecithin would enable the membrane lipids to reform virtually continuous bimolecular leaflets in stage 5.

Although these diagrams illustrate a relatively simple situation in which the transition from a bimolecular leaflet to a micellar configuration results from the incorporation of perturbing molecules into the lipids of the membrane, I anticipate that different kinds of perturbing molecule will produce comparable changes in the organization of membranes by differing mechanisms. Some of these may involve interactions not only with the proteins but also with the glycoproteins of membranes. (The diagrams of Fig. 2 are necessarily a gross simplification.)

Any comprehensive molecular mechanism for membrane fusion must also take into account the known energy requirements for fusion. Although the discharge of zymogen granules is independent of protein synthesis, the process nevertheless uses respiratory energy¹⁶. Formation of very small liposomes by prolonged ultrasonic dispersion of lecithin in water, and the production of microsomal vesicles from the endoplasmic reticulum by homogenization, may result from the pinching-off of membranes and not simply from the linking of the ends of ruptured fragments. Should this be so, the applied sonic or mechanical energy may be responsible for the occurrence of transient phase changes in the membrane lipids which facilitate membrane fusion. Energy may

also be needed in stage 5 of the fusion process. Respiratory energy is apparently required for the fusion of Ehrlich ascites cells by Sendai virus. This may be related to the stability of the membranes of newly formed polykaryocytes, for cell lysis occurred instead of cell fusion when 2,4-dinitrophenol was added before incubation of the cells with virus at 37° C (ref. 7). Control cells were not lysed by dinitrophenol. On the present hypothesis, these observations may be interpreted in terms of cell fusion occurring up to and including stage 4 but with lysis occurring in the presence of dinitrophenol as a result of a failure of metabolism to convert extensive areas of micellar configuration back to the bimolecular leaflet form in stage 5. Should cell fusion induced by Sendai virus involve lysolecithin, the requirement for respiratory energy may be for the acylation of lysolecithin to lecithin in stage 5. This reaction, which occurs in intact and lysed erythrocytes, requires coenzyme A and ATP¹⁷.

Lysolecithin

Lysolecithin constitutes one of the simplest agents that would be expected to facilitate membrane fusion. As a consequence of its wedge shape, the incorporation of lysolecithin into a bilayer should produce a transition from a bimolecular leaflet to radially orientated molecules⁸. When lysolecithin is dispersed in water it forms globular micelles¹⁸ and the effectiveness with which lysolecithin micellizes membrane lipids¹⁹ is probably responsible for the lytic properties of this molecule.

Lysolecithin will induce cell fusion and heterokaryons can be produced by the use of lysolecithin⁶. It is unlikely, however, that lysolecithin causes membrane fusion solely by an effect on membrane lipids. Lysolecithin seems also to interact with the proteins of membranes. It has been reported that treatment of myelin with lysolecithin yields a complex protein-lipid-lysolecithin micelle²⁰. Nuclear magnetic resonance studies of erythrocyte membranes co-dispersed with lysolecithin also indicate that lysolecithin forms an additive complex with membrane material²¹, and work with optical methods suggests that treatment of plasma membranes with lysolecithin or with phospholipase A results in changes in the structure of membrane proteins²². In the light of these findings, the simple diagram of Fig. 2:3 may be modified to indicate a possible role for membrane proteins in the fusion of cells that is induced by lysolecithin (see Fig. 3).

Vitamin A Alcohol

Retinol resembles lysolecithin in behaving as a surface-active agent^{23,24}. It causes leakage of enzymes from isolated lysosomes *in vitro*²⁵ and the loss of haemoglobin and potassium from erythrocytes²⁶. Retinol would be expected to decrease the stability of a bimolecular leaflet of phospholipid⁸, so that it has been proposed that the lytic actions of retinol arise from the presence of a high proportion of globular lipid micelles in membranes that contain an excess of retinol⁴. On the present hypothesis, retinol (like lysolecithin) should cause membrane fusion, and experiments are being undertaken in this laboratory to see if retinol will cause cell fusion and the formation of syncytia. It has already been demonstrated, however, that treatment of erythrocytes with retinol leads to invagination of the cell membrane, followed by the formation of internal vacuoles by a process morphologically resembling pinocytosis²⁷. The membrane fusion that is involved in this process may be depicted by Fig. 2:2 if both of the membranes in this figure are regarded as micellar portions of the infolded plasma membrane of the cell. Interdigitation of the micelles (Fig. 2:3) would lead to the separation of the interior of the newly-formed vacuole *Z* from the exterior of the cell *Y*. With retinol, formation of internal membrane-bound vacuoles is associated with a general increase in the permeability

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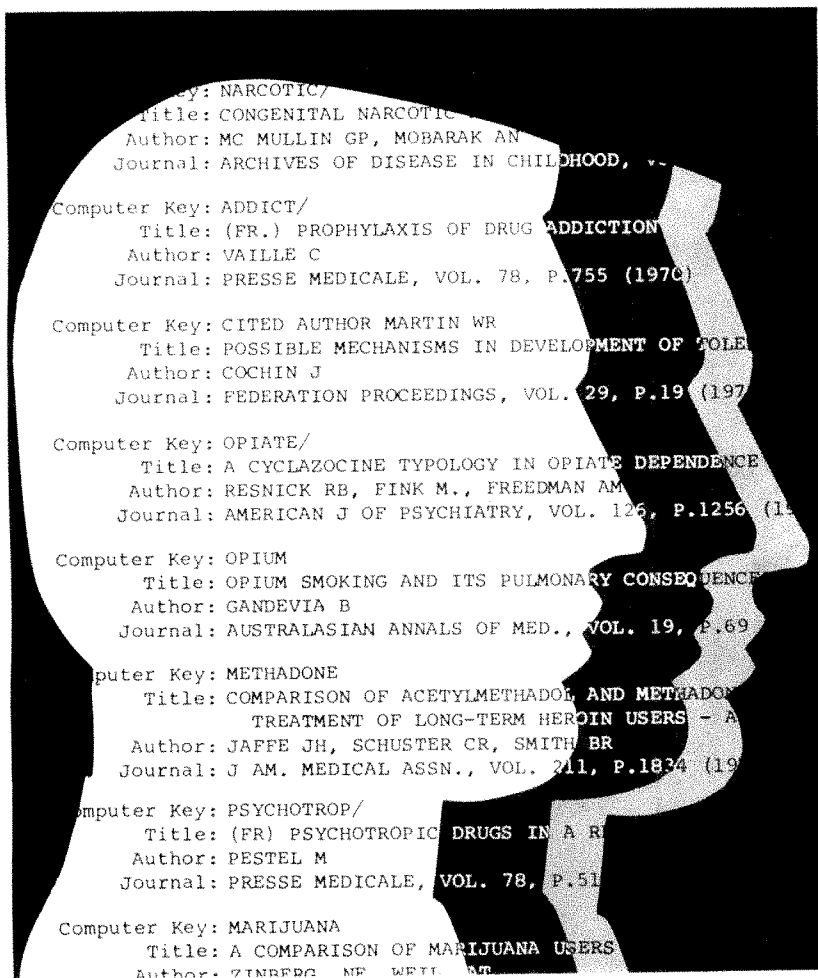
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of the plasma membrane and subsequent cell lysis. By contrast, agents such as polyamino-acids, which induce pinocytosis apparently without increasing membrane permeability²⁸, may facilitate a transition to the micellar configuration at fewer more localized sites by specific interactions with membrane proteins.

In living chondrocytes, vitamin A facilitates the fusion of some form of lysosome—possibly enzyme-containing Golgi vesicles—with the plasma membrane. Retinol causes an extracellular release of lysosomal enzymes from skeletal tissue in organ culture, and this leads to a degradation of the extracellular matrix of cartilage rudiments^{29,30}. There is no biochemical or cytological evidence of cellular damage when this occurs, and it seems unlikely that large quantities of lysosomal enzymes are freely released within the cells. This action of retinol may perhaps be explained by proposing that it forms lipid micelles in the plasma membrane, and facilitates the fusion of lysosomal membranes (that may already contain micelles because of the presence of lysolecithin) with the plasma membrane (see Fig. 2). A similar mechanism may conceivably apply to the fusion of primary lysosomes with the plasma membrane by complement-sufficient antiserum, which causes a specific synthesis and release of lysosomal hydrolases into the extracellular environment without the apparent release of lysosomal enzymes within the cell³¹. Complement-sufficient antiserum seems to have no effect on isolated lysosomes^{32,33}.

General Considerations

Many biological phenomena involve membrane fusion, including fertilization, cell division, extracellular secretory processes, endocytosis, the formation of secondary lysosomes, the extracellular release of enveloped viruses, and cell fusion occurring *in vitro* or *in vivo* either as a result of a virus infection, or spontaneously, to yield heterokaryons or normal multinucleated cells such as myotubes. Some general mechanism for membrane fusion may apply, with variations in individual systems, to all these processes. The mechanism proposed here for membrane fusion is too rudimentary to warrant an extended discussion of its possible application to these various phenomena, but it is interesting to consider certain features of secretion in the present context. Fusion of subcellular organelles with the plasma membrane seems, in many cases, to be the result of a complex sequence of events. Thus a number of stages are distinguishable in the glucose-stimulated secretion of insulin, and glucose does not cause the direct release of insulin from isolated secretory granules³⁴. By contrast, pinocytic vacuoles commonly fuse with one another to form larger vacuolar structures^{35,36}. Some secretory organelles, for example, chromaffin³⁷ and zymogen granules³⁸, are capable of fusing with one another. Secretory granules in the anterior pituitary gland also fuse with lysosomes when extracellular secretion is suppressed³⁹. Thus while fusion of organelles with the plasma membrane seems to depend on the occurrence of an induced change in the structure of the plasma membrane, the observed inter-organellar fusion of secretory and digestive vesicles may indicate the existence of a common structural organization in the membranes of these organelles which facilitates fusion.

Factors inhibiting membrane fusion, although not considered in detail here, may be regarded as of equal importance to those facilitating fusion. It is noteworthy that high concentrations of calcium inhibit the extrusion of lysosomal enzymes from leucocidin-treated leucocytes⁴⁰. Degranulation of leucocytes is particularly interesting, for the inhibition of degranulation produced by high levels of calcium may possibly arise from an inhibition of micelle formation, which could result from cross-linking by divalent calcium ions of adjacent phospholipid molecules in a lipid bilayer¹⁴. Another agent known to stabilize membranes is cortisol. Attention has previously

been drawn¹⁵ to the fact that the action of retinol in producing both instability in individual membranes and facilitating membrane fusion is matched by a similar parallelism for cortisol, which stabilizes membranes and inhibits membrane fusion.

In summary, I suggest that the interaction and intermixing of the lipids of membranes which occur during fusion require the lipid molecules of both membranes to be appropriately organized; and further, that agents capable of causing membrane fusion do so by inducing—directly or indirectly—a phase change in the lipids of one or both of the membranes concerned. For fusion to occur a relatively high proportion of the lipids of both membranes may need to be organized in micelles of lipid or lipoprotein. Interdigitation of the micelles of the two membranes could then lead to membrane fusion. On this hypothesis, any structural, metabolic or energetic factor that acts on the lipid, protein or glycoprotein moieties of membranes and favours the micellar configuration in local areas should facilitate the fusion of membranes at these sites. Certain agents such as lysolecithin would therefore be predicted to induce fusion, and support for the proposed mechanism is provided by experiments in which lysolecithin has been observed to cause cell fusion⁶. This working hypothesis may be of value in future experimental studies, particularly those on the control mechanisms involved in fusion in biological systems.

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X-ray Photographs of the Sun on March 7, 1970

by

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The Sun was photographed from an Aerobee rocket by means of equipment responding to the wavelength interval 3–60 Å, revealing interesting relationships between structures in the corona.

DURING the eclipse of March 7, 1970, we obtained X-ray photographs of the Sun with an Aerobee rocket payload. The photographs were taken at the end of the partial eclipse as seen from White Sands, New Mexico. X-rays in the wavelength region of sensitivity of our telescope (3–60 Å) are emitted essentially only at temperatures higher than 10^6 K. The X-ray images therefore show the solar corona without interference from the radiation of the cooler photosphere and chromosphere, and coronal structures on the disk as well as at the limb can be studied.

The purpose of this preliminary article is to present a morphological description of the X-ray corona of March 7 and to relate the X-ray observations to the white light observations of the limb¹ and to the observations of the photospheric magnetic field² on March 7. This preliminary discussion is limited to the large scale X-ray structures and their most evident associations with the magnetic field and the white light corona. The quantitative analysis of the X-ray photographs, their spectral content, and the detailed association of the X-ray emission with the magnetic field structures and with the structures seen at other wavelengths, will be treated in later articles.

In previous papers^{3–5} we have stressed the importance of the photospheric magnetic field in determining the three-dimensional structure above the active regions. This new set of photographs also demonstrates the magnetic field to be the governing agent for the structure of the X-ray emission outside the active regions. In particular: (1) We establish the presence in the corona, as seen in X-rays, of a "complex of activity" and relate it to the definition of Bumba and Howard⁶. (2) We establish the presence at coronal heights of the interconnection of the preceding polarities of active regions across the solar equator as originally postulated by Babcock⁷. (3) We identify certain diffuse components of the X-ray corona outside active regions with old remnants of former active regions and relate them to the unipolar magnetic regions of Bumba and Howard and their "ghosts", and therefore to the bases of the white light rays and helmet streamers. (4) We recognize several components in the X-ray emission of a plage including a small bright component located in regions of large longitudinal field gradient which is similar in appearance to X-ray flare features that we have observed in previous flights.

Instrumentation and Data

The X-ray telescope is very similar to the one flown over the past several years and described in detail in the literature^{4,5,8}. It consists essentially of a grazing incidence X-ray mirror, a camera in the focal plane, a sequence of filters transmitting specific broadbands in the 3–60 Å wavelength region, a pointing control system and appropriate control electronics. Three years of minor but important improvements in the instrumentation (particularly in the areas of filters, camera and electronic controls) have led to higher sensitivity for the longer wavelength X-rays which are produced primarily by the cooler coronal plasma. Faint structures in the corona outside active regions which would have been unobservable in previous flights are therefore visible in these photographs. These results clarify the association of the X-ray corona with chromospheric features and the white light corona.

The data consist of a series of exposures with various passband filters and exposure times (Table 1).

Exposure No.	Duration* (s)	Nominal passband†
1–62	1.5	P, 3–30 Å, 44–55 Å
63	49	P, 3–30 Å, 44–55 Å
64	29	M, 3–15 Å, 44–48 Å
65	27	T, 3–13 Å, 19–24 Å
66	11	P, 3–30 Å, 44–55 Å
67	6	T, 3–13 Å, 19–24 Å
68	6	M, 3–15 Å, 44–48 Å
69	7	B, 3–15 Å
70	1	White light

* The exposures were taken between 1854.28 and 1858.54 UT.

† The filter materials are as follows: P = 0.85 µm 'Parylene N' + 2500 Å Al; M = 3.8 µm 'Mylar' + 3000 Å Al; T = 3.2 µm Teflon + 1.1 µm 'Parylene N' + 3000 Å Al; B = 1.3 µm Be + 1000 Å Al. The nominal passbands correspond to 5 per cent or greater transmission.

Description of Photographs

The general appearance of the X-ray corona is similar to that observed in our previous experiments^{3–5}, but the higher sensitivity at longer wavelengths (due to filters of greater transmission) results in a more complete view of the various coronal X-ray structures and of their relation to the photospheric magnetic field, and the chromospheric features.

Figs. 1a and b (exposures 66 and 65 of Table 1) show the X-ray corona in different wavelength passbands. The combination of passbands and exposure times is such that Fig. 1a shows the more diffuse component of the corona while Fig. 1b shows structures within the active regions. The limb of the Moon is evident near the east limb of the Sun. Diffuse X-ray emission, often having a loop-like appearance, covers the disk intensely in the regions of the activity belts and weakly in the polar regions. The limb brightened X-ray emission around the Sun is not uniform and local regions of enhanced X-ray emission and gaps of reduced emission are present. This is most noticeable in the north and south polar regions where there is less interference from the emission of active regions on or near the limb. Near the active regions, close to the limb, there are structures looping above the active regions and arching from one active region to another. This is particularly noticeable in the complex of active regions at the west limb, where structures link McMath regions 10621, 10602, 10604, 10595 and 10598 (an identification of the corresponding CaK plages is given in Fig. 2). The features on the north central portion of the disk would presumably have a similar appearance if observed at the limb. In Fig. 1b the X-ray structure of the active regions is evident. Most X-ray plages contain one or more bright, narrow cores, typically 10 to 20 arc seconds in length and having a striated appearance with apparent width comparable with the resolution of the X-ray telescope (3 to 5 arc seconds). These are particularly noticeable in regions 10618A, 10618B, 10618C, 10618D, 10614, 10617 and 10607 (see Fig. 2). A more extended emission involving the overall plage and revealing its three-dimensional structure is seen superimposed on the bright cores.

Fig. 1c is a composite of the exposure shown in Fig. 1a and the white light corona as photographed during the eclipse by Newkirk and Lacey¹ of the High Altitude Observatory. There is a detailed correspondence between various features on the X-ray exposure and white light coronal structures. At the limb, the X-ray brightenings noted in Fig. 1a mark the bases of the white light coronal features. Table 2 describes the relationship between the white light and X-ray appearance of some of the more notable coronal features.

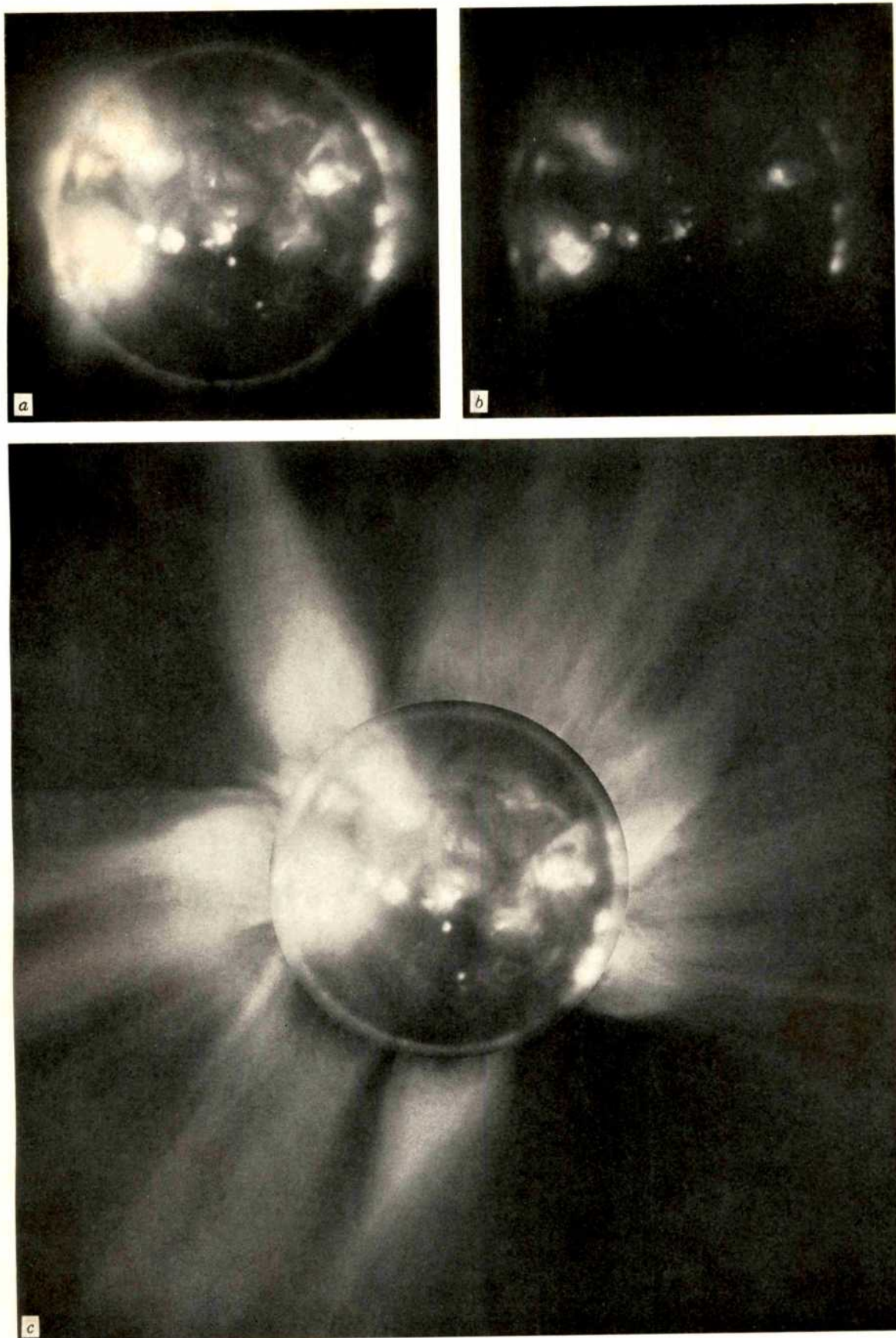


Fig. 1. The appearance of the corona in X-rays and white light on March 7, 1970. *a*, An X-ray exposure in the bands 3–30 Å, 44–55 Å; *b*, an exposure in the band 3–13 Å, 19–24 Å; *c*, a superposition of the X-ray exposure of *a* and the white light coronal photograph taken by Newkirk and Lacey¹.

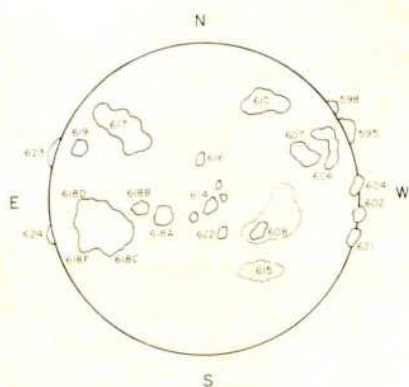


Fig. 2. A schematic drawing of the solar disk of March 7, 1970, identifying active regions by their McMath plage numbers.

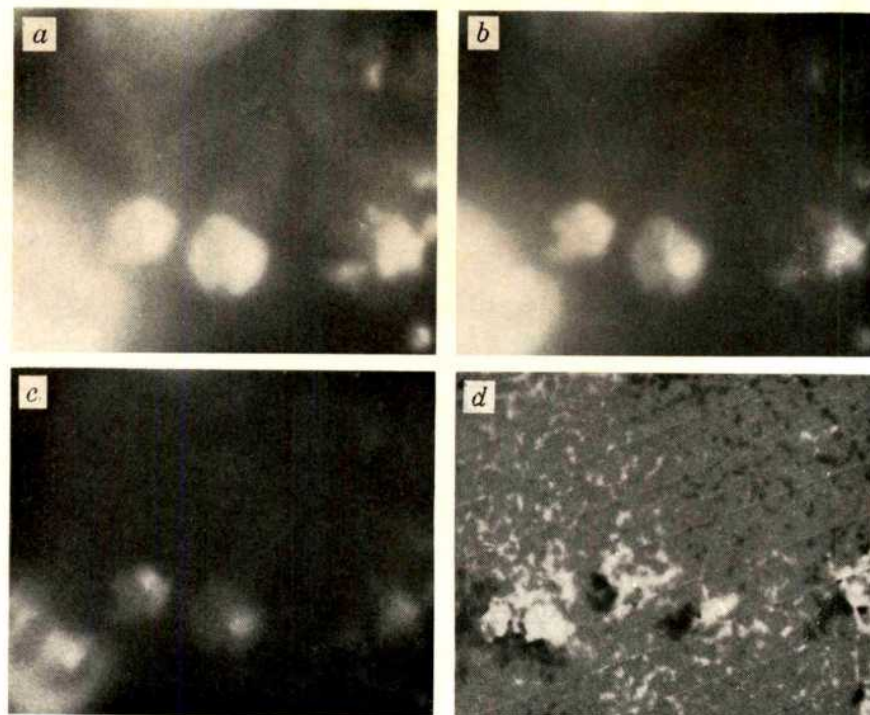


Fig. 3. Comparison of X-ray emitting structures as photographed in three passbands: (a) 3-30 Å, 44-45 Å; (b) 3-15 Å, 44-48 Å; (c) 3-13 Å, 19-24 Å; and (d) the corresponding photospheric magnetic field, measured by Livingston *et al.*⁵

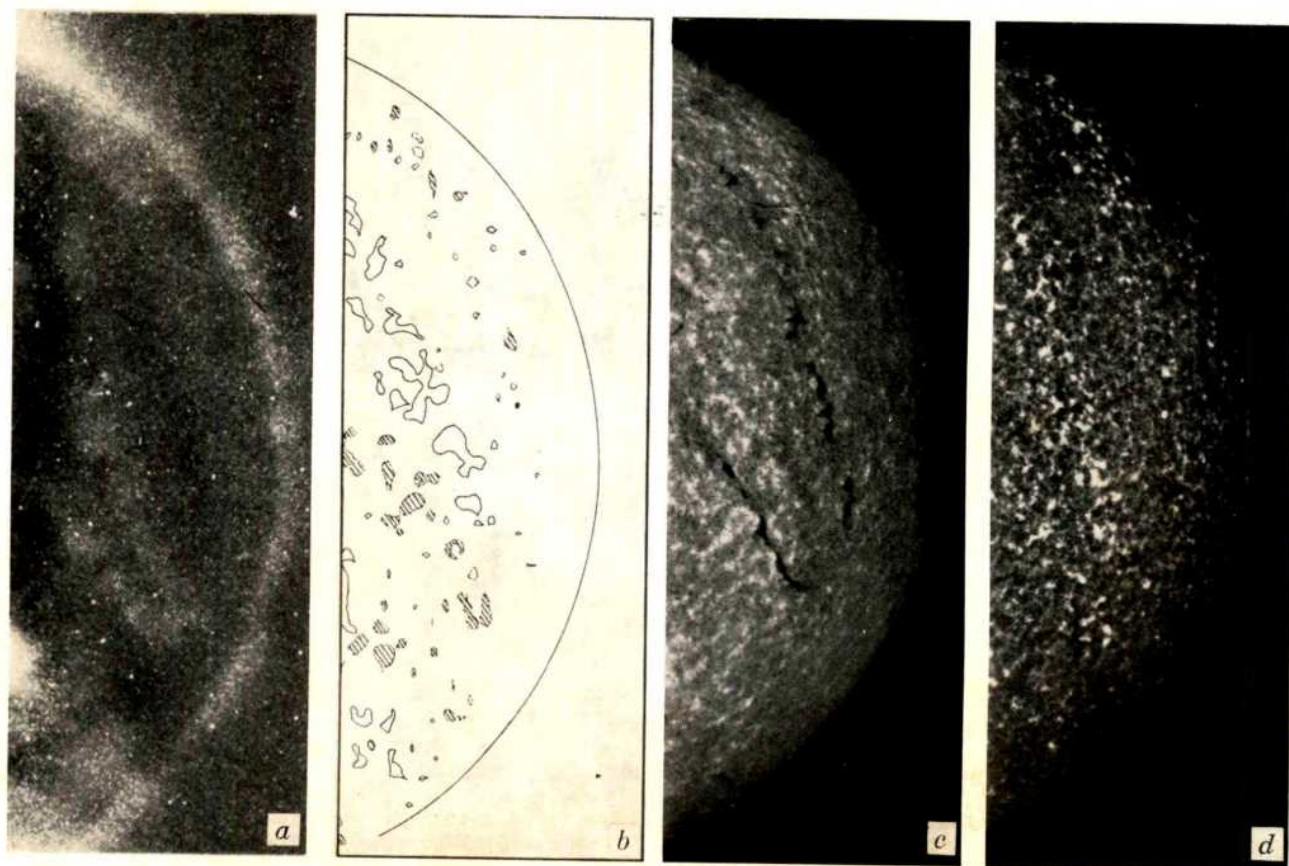


Fig. 4. A region of diffuse X-ray emission on the disk as observed on November 4, 1969, in (a) soft X-rays, (b) photospheric magnetic fields, (c) H α , and (d) CaK. The photospheric magnetic field measurements were provided by Mount Wilson Observatory; the H α and CaK photographs were provided by Sacramento Park Observatory.

Table 2. RELATIONSHIP OF WHITE LIGHT AND X-RAY FEATURES

Position angle (measured east from solar north)	Remarks
50°	The large helmet streamer observed in white light cannot be regarded as an extension of the X-ray structure above the core of region 10617. We therefore infer that this streamer must be associated with the filament and large scale active region remnants to the east. The relationship between region 10617 and these remnants is clearly shown by the magnetogram of March 12, 1970, obtained by Livingston <i>et al.</i> ²
80°	The bright enhancement at the X-ray limb corresponds to a coronal white light feature. We attribute these features to region 10623, east of the limb on March 7.
98°	The enhancements at the X-ray limb and the associated white light helmet streamer are not connected to region 10618, but originate behind the limb probably in region 10624 or related remnants.
140°-175°	There is a one to one correspondence between the bright limb X-ray enhancements and the brighter regions at the base of the assembly of white light streamers.
180°	A gap in the bright X-ray limb corresponds to the inter-streamer region visible in white light.
194°	The white light helmet streamer is not associated with any front disk X-ray feature. X-ray enhancements are again apparent at the bases of the streamer. The streamer is probably associated with the remnants of region 10584 on the other side of the Sun as conjectured by Teske <i>et al.</i> ³
215°-240°	A large gap appears in the bright X-ray limb associated with the white light features of much lower intensity in this region. Faint X-ray emission from the limb is evident near the middle of this gap on the original negatives however.
250°	A complex X-ray structure is evident on the limb inter-connecting regions 10621, 10602 and 10604. The general shape of this structure corresponds to the shape of the lowest portions of the white light streamer.
292°	The bright "horn" in white light is associated with X-ray emission corresponding to regions 10595 and 10598 behind the west limb. The X-ray structures also indicate a connexion to region 10604 to the south which may explain the nonradial appearance of the white light structure.
330°-30°	As in the south-east, there is an excellent correspondence between brightness enhancements on the X-ray limb and the bases of the white light rays.

In Fig. 3, enlargements of a section of the disk, photographs in different wavelength passbands, are compared in detail with the magnetic field. Fig. 3a is the exposure shown in Fig. 1a. Fig. 3b is a 29 s exposure in the nominal passbands 3 to 15 Å and 44 to 48 Å. Fig. 3c corresponds to Fig. 1b and Fig. 3d is part of the magnetogram of Livingston *et al.*². There is an evident relationship between the coronal structures observed in soft X-rays and the underlying photospheric magnetic field. The diffuse X-ray emitting regions running north-south in Fig. 3a connect regions of preceding magnetic polarity across the solar equator. Both the general X-ray plage structure, seen best in Fig. 3b, and the bright X-ray plage cores of Fig. 3c seem to take the form of tubes linking the preceding and following magnetic polarity portions of the active regions. The bright, narrow tubes appear where the gradient of the longitudinal photospheric magnetic field is highest and connect adjacent regions of opposite polarity. The larger, dimmer tubes follow more curving paths and connect more widely separated regions.

Interpretation of Results

From the general appearance of the X-ray corona on the disk we observe that large sections of the solar atmosphere encompassing many active regions and remnants of older regions are interconnected by emitting loops filled with plasma. From the comparison with the magnetic field maps of Livingston *et al.*² and the definition and discussion of Bumba and Howard⁶, it is apparent that they are the coronal configurations corresponding to their "complex of activity". The best example shown in Fig. 1a includes active regions 10614, 10618A, 10618B, 10617 and 10619. Notice the lack of emission separating this complex from the other complex including 10606, 10607, 10608 and 10610; in H α this separation is marked by an old filament.

The active regions imbedded in a "complex of activity" show a general X-ray structure, seen best in Fig. 3b,

which often seems to take the form of tubes linking the preceding and following magnetic polarity portions of the active regions. Often active regions are "mixed" in the sense that a considerable portion of the magnetic flux of a following polarity is linked to a preceding polarity portion of another active region. The sharing of flux between 10618A, B and C in Fig. 3a is a clear example of this. When seen close to the limb these active region structures occupy the cores of hot coronal condensations and young streamers as seen in white light (see in Fig. 1c the regions on the west limb and the regions corresponding to plage 10623).

Within this general X-ray structure most active regions contain very bright structures, often striated and tubular in appearance, linking the preceding and following magnetic polarity of the region across the neutral line of the longitudinal field at points where the gradient of the longitudinal field appears to be particularly high. The positional association with the neutral longitudinal field line, where many flares are known to occur, and the fact that the flare we observed on June 8, 1968, had a bright hot kernel associated with one such feature suggest that the small bright active region structures play an important part in the X-ray flare. The data are also consistent with a continuous distribution of loop configurations in which the vertical scale of a structure reflects the horizontal separation of the connected regions, and structures linking widely separated regions are higher, less dense and therefore weakly emitting, whereas the structures connecting adjacent regions of opposite polarity are lower, more dense, and therefore strongly emitting.

Among the diffuse emission linking active regions in a "complex of activity" we find several examples of X-ray emission showing magnetic flux going from preceding polarities on one hemisphere to preceding polarities on the other hemisphere. A good example is shown in Fig. 3a where the preceding polarity portion of regions 10618A and B connect to remnants of opposite polarity in the north and toward the preceding portion of 10617. This coronal configuration is consistent with a "severing and reconnection of lines of force" of the type required by the Babcock model of the solar cycle⁷.

We have previously^{3,4} pointed out the presence of weak X-ray emitting regions associated with the brighter portions of the calcium network pattern. We have also argued from the similarity of the spectra of the X-ray corona seen at the limb and of the diffuse emission on the disk that these are two views of the same object. A series of four rocket flights by our group over the past three years have (1) confirmed the existence of the diffuse component; (2) shown that these structures are more diffuse than the calcium network although they often have a feather-like appearance at our resolution; (3) identified the brightest diffuse structures, if not all of them, with remnants of old active regions of the type giving rise to unipolar magnetic regions and their "ghosts"⁸; and (4) related them to enhancements at the X-ray limb. A good example of these enhanced diffuse emissions on the disk is shown in the X-ray photographs taken on November 4, 1969, with similar instrumentation. Figs. 4a, b, c and d show the appearance of the south polar region in X-rays, magnetic field, H α and CaK respectively. Notice the filament separating the regions of opposite polarity and the corresponding gap of emission in the X-ray photograph.

The eclipse X-ray observations and their correlation with the white light corona permit us to associate the feet of coronal rays and helmet streamers with X-ray enhancements at the limb (Fig. 1c). This, together with our previous observations, allows the association of X-ray diffuse structures on the disk with streamers and rays. This is in agreement with the evolutionary classification of helmet streamers by Newkirk¹⁰ (based on the inferred association of prominences and migrating magnetic regions) and with our interpretation of the diffuse X-ray

enhancements on the disk with old remnants of active regions.

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Interstellar Silicate Absorption Bands

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Interstellar silicate absorption is observed in the direction of the galactic centre. Most interstellar silicon seems to be in silicate form.

SILICATES have two absorption bands near 10 μm and 20 μm . Fig. 1 from Lyon¹ shows the absorption spectrum of enstatite ($\text{Mg}_{0.5}\text{Fe}_{0.5}\text{SiO}_3$), a typical meteoritic mineral. Because Knacke, Gaustad, Gillett and Stein² had apparently observed a 10 μm interstellar absorption in the M21b supergiant 119 Tauri, we looked for more heavily reddened objects in which the absorption should appear in medium bandwidth photometry. Unfortunately the brightest reddened objects at these wavelengths, M supergiants, have large unpredictable infrared emission where the absorption feature should appear.

Reddened B stars might provide a more predictable continuum, and so three B stars with about four times the colour excess of 119 Tauri were observed. A four-colour photometer was used with the Kitt Peak 50 inch metal mirror telescope. The photometric equipment constructed and assembled by Drs Gillett and Stein provided passbands at 3.8, 4.8, 8.5 and 11.5 μm effective wavelength. At 3.8 μm we have allowed for atmospheric water vapour in assessing the effective wavelength.

The two longest wavelength passbands can be compared with Fig. 1, where it is seen that silicates have little absorption at 8.5 μm , but peak absorption near 11.5 μm . The observations of the stars are reported in Table 1, together with visual observations by Hiltner³.

First we note that for unreddened stars of type near AO, all magnitudes should be approximately the same. Unfortunately the difference between the 8.5 μm and 11.5 μm magnitudes for the first two stars is about equal to the uncertainty in these differences. In HD 168625, the elevated continuum suggests that the star is a Be star, and uncertainties about the shape of the continuum prevent these observations from being used. Clearly, reddened B stars are both rather faint for this study and also have unpredictable continua.

Dr F. J. Low has suggested (personal communication) that the galactic centre provided a bright continuum in the 10 μm region while suffering severe visual interstellar absorption. Further, he commented that his

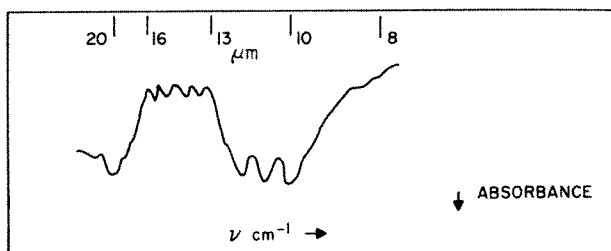


Fig. 1. Absorption spectrum of enstatite ($\text{Mg}_{0.5}\text{Fe}_{0.5}\text{SiO}_3$).

photometric measures⁴ appeared to indicate spectral structure in the 10–20 μm region.

Four-colour photometric measures were then also made for the galactic centre by R. D. G. and N. J. W. The 10 inch diameter photometer beam was centred on the source by peaking the signal observed at 11.5 μm . Atmospheric absorption corrections were made by observing α Sco at the same air mass. This star is well tied to our photometric system, and the shape of the 10 μm continuum is also known from spectral scans. The results for two separate nights of observation are shown in Table 2.

Table 2. PHOTOMETRIC OBSERVATIONS OF THE GALACTIC CENTRE WITH 10" BEAM

Night	Scale	3.8 μm	4.8 μm	8.5 μm	11.5 μm
June 23, 1970	Magnitude	+5.04	+3.01	-0.91	-1.94
June 24, 1970		+4.19	+2.04	-1.19	-2.02
June 23, 1970	Flux units	3.1	12.6	150	210
June 24, 1970		6.8	30.6	195	224

It is believed that the short wavelength discrepancies between the two nights are caused by slightly different centring of the beam, combined with the wavelength-dependent source structure and telescope diffraction. At 3 μm Becklin and Neugebauer⁵ have shown that a

Table 1. PHOTOMETRIC OBSERVATIONS OF REDDENED B STARS

Star	Type	V	3.8 μm	4.8 μm	8.5 μm	11.5 μm	$\Delta(\text{B}-\text{V})$	$\Delta(\text{V}-8.5)$
HD 183143	B7Ia	+6.87	+3.22	+3.11	+2.73	+2.95	1.29	4.14
168607	B9I*ap	+8.29	+3.15	+2.42	+2.56	+2.77	1.60	5.73
168625	B8Ia+*	+8.41	+3.82	+3.01	+1.80	+1.14	1.48	6.81

* $\text{H}\beta$ is suspected to be in emission in this star, noted in HD catalogue.

point source dominates the energy output while at $10\ \mu\text{m}$ the core has an angular extent of 15 inches, slightly displaced from the point source. If either set of measures are plotted as magnitude against $1/\lambda$, the 11.5 points seem to fall below a smooth continuum by ~ 0.5 .

A better measure of the depth of the interstellar feature can be obtained by combining the present observations with those of Low *et al.* Fig. 2 shows the two sets of observations normalized to a common beam size by assuming that all fluxes measured by Low *et al.* have the same angular distribution as the $11.5\ \mu\text{m}$ flux. If a smooth continuum is drawn, there seem to be two absorption features with a similar wavelength distribution to Fig. 1. The central optical depth of the band at $10\ \mu\text{m}$ is $\tau \approx 0.7$. Gaustad⁶ has tabulated the absorption coefficient of enstatite, and the observations would require $3 \times 10^{-4}\ \text{g cm}^{-2}$ of silicate to produce the observed absorption. The visual absorption of the galactic centre is 25 ± 5 magnitudes, thus there seems to be $1.2 \times 10^{-5}\ \text{g cm}^{-2}$ of silicate per magnitude of visual absorption. At a typical galactic plane absorption of $2\ \text{mag kpc}^{-1}$ and interstellar hydrogen density of $1\ \text{atom cm}^{-3}$, silicates would form 0.4 per cent by mass of the interstellar medium. For typical cosmic abundances, this is the maximum amount of silicate that could form, being limited by the cosmic silicon abundance.

The star 119 Tauri suffers ~ 1.5 of visual absorption, and thus by comparison with the galactic centre one would expect a central depth of the $10\ \mu\text{m}$ feature of only 4 per cent, which is undetectable with present accuracy. Other doubts about this observation have also arisen⁷. The spectrum of 119 Tauri was therefore observed at Kitt Peak by J. A. H. and N. J. W. The spectrometer was identical with that used at Lick Observatory for the earlier observations. A method of rapid scanning was used, and after 8 h of observation and 178 scans, the results seemed to show a significant discrepancy with the earlier measures in that the $9.6\ \mu\text{m}$ feature did not appear, Fig. 3. Despite the inadequate extent of the spectrum to short wavelengths, however, the SiO double feature at $8.1\ \mu\text{m}$ centre does appear. There is a possible wavelength discrepancy of

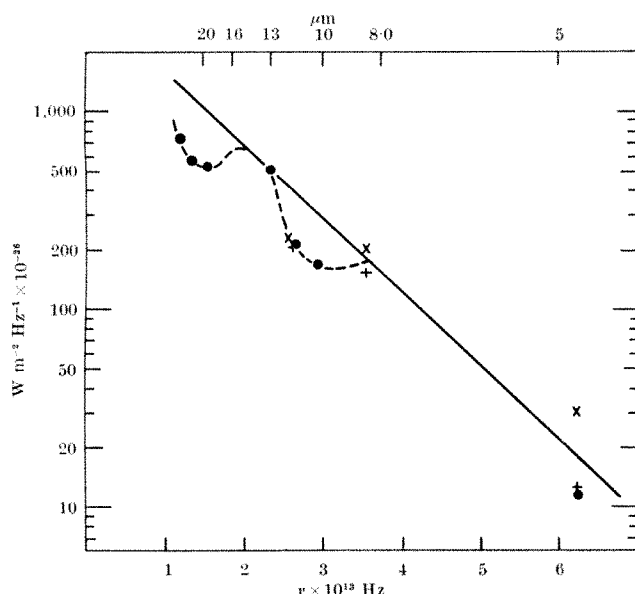


Fig. 2. Photometric measures of the galactic centre. ●, Low *et al.*; +, night 1; x, night 2.

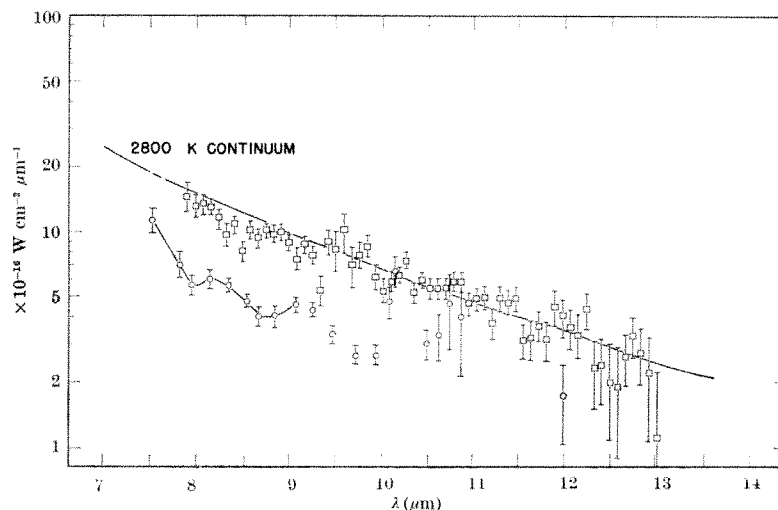


Fig. 3. Spectrum of 119 Tauri (M2Ib). □, Hackwell; ○, Knacke *et al.*

$0.1\ \mu\text{m}$ between the two observations, and because greater care was paid to this point in the earlier observations, this should not be regarded as a significant difference. The difference in absolute calibration, however, is significant and is perhaps related to the different energy distributions derived in the two observations.

It seems that the earlier results may have suffered from inadequate correction for ozone absorption. Large telescopes are extremely efficient for spectral studies, as the observing time required is proportional to (diameter)⁻⁴; however, because of limited access to large optical telescopes, there has been a tendency to spend most time observing faint sources, and insufficient observing time has been spent tying down atmospheric corrections. One of us (N. J. W.) assisted in the planning and execution of some of the earlier observations of 119 Tauri.

In contrast to the discrepancy between the galactic centre observations and 119 Tauri earlier observations, the observations of the reddened B stars are consistent with the galactic centre observations. The stars HD183143 and HD168607 would be expected to show a deficiency of $11.5\ \mu\text{m}$ over $8.5\ \mu\text{m}$ by $+0.16$. The agreement with the observed value $+0.23$ is much better than the observational error would predict.

In the agreement with expectations aroused when silicates were identified in the Orion Nebula^{8,9}, this material is abundant in interstellar space. The abundance seems to be limited by the cosmic silicon abundance. The absorption spectrum seems to be typical of terrestrial silicates and not anomalous as might have been inferred by a premature report of its discovery.

We thank Dr G. Neugebauer for providing a finding chart for the galactic centre. Generous provision of observing time by Kitt Peak National Observatory made these observations possible. J. A. H. is supported by a special grant from the Science Research Council of Great Britain; R. D. G. is a US National Science Foundation trainee. This research was supported by funds from NASA and by the Graduate School, University of Minnesota.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Direct Method of Measuring Stratospheric Water Vapour Mixing Ratios

THE measurement of the mixing ratio, μ , of water vapour to air in the stratosphere has been carried out many times, using spectroscopic methods, from both aircraft^{1,2} and balloons³⁻⁶. As a result of the range of values that have been obtained the subject has become controversial^{6,7}. We present here some submillimetre wavelength measurements made in an aircraft observing atmospheric emission, using methods of Fourier transform spectrometry⁸, which may provide an improved method of obtaining this parameter. Previous submillimetre atmospheric emission measurements⁹ have produced estimates of stratospheric water content, but the quantitative value of these results is limited because of the problem of radiometrically calibrating the system in order to obtain reliable measures of background and zero levels. In the present work the direct comparison of water vapour and oxygen emission has largely removed these requirements and greatly simplified the analysis.

The observations were made flying in a Comet 2E of Radio Flight, RAE, Farnborough, England, on November 14, 1969, at an altitude of 12.2 km, with an ambient temperature of 222 K (at 2.8 km above the tropopause, temperature 215 K). (These data were supplied by the Meteorological Office, Bracknell, Berkshire.) A Michelson interferometer and a 350 mm diameter Cassegrain telescope were mounted at an angle of 10° above the horizontal to view the atmosphere through a four-aperture, 16 mm thick, polypropylene window. The submillimetre radiation exchange passing between the Golay cell detector and the atmosphere was phase-modulated (J. Chamberlain, unpublished) in the interferometer, amplified, synchronously rectified, and the output signal digitally recorded after smoothing with a 1 s time constant electronic filter. The greatly improved performance

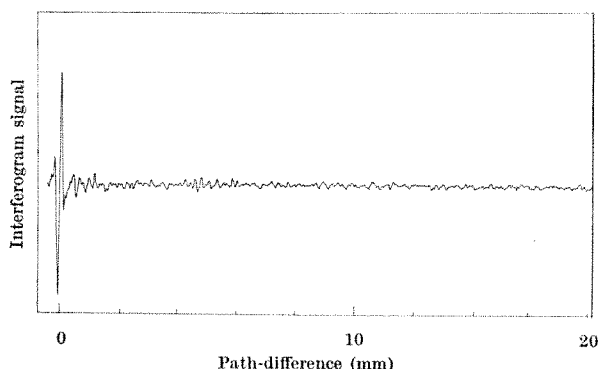


Fig. 1. Phase modulated interferogram function obtained at an altitude of 12.2 km.

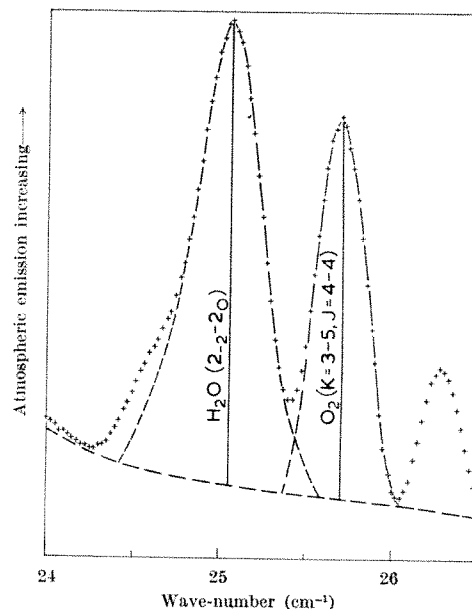


Fig. 2. Example of observed atmospheric emission used to estimate the water vapour mixing ratio μ . The crosses are the computed spectral points and the dashed lines show the measured emission areas. (Average of two spectra; altitude 12.2 km; resolution 0.5 cm⁻¹.)

gained by phase-modulation (J. Chamberlain, unpublished) resulted in interferograms with a signal to noise ratio about 40 (Fig. 1) and, from these, good quality transformed spectra over the range 20 to 65 cm⁻¹ were obtained to a resolution limit of 0.5 cm⁻¹ in an observation time of 30 min. At this resolution limit a large number of atmospheric features can be identified, and the analysis of these, together with a complete discussion of experimental details, will be published later. Here we concentrate on the features assigned to water vapour and oxygen and, in particular, pairs of comparable intensity and separated by only a small frequency interval (< 2 cm⁻¹). An example of two such lines, occurring in the 25–26 cm⁻¹ region, is shown in Fig. 2. These features can be used to make a direct measurement of the mixing ratio of water vapour and oxygen, and hence of water vapour to air (μ) with knowledge of the oxygen to air mixing ratio.

The computation of the mixing ratio μ is possible if it can be assumed that over the emission path the water vapour to oxygen mixing ratio is constant and the temperature of the region is approximately constant. Then if the emission lines are "strong" (that is, with an emissivity of the order of unity in the region of line-centre), the ratio of the integrated emission-strengths (E) of the two lines can be shown to be given by¹⁰

$$\left(\frac{E_{\text{H}_2\text{O}}}{E_{\text{O}_2}}\right) = \left(\frac{S_1 \Delta\sigma_1 p_1}{S_2 \Delta\sigma_2 p_2}\right)^{\frac{1}{2}}$$

so that

$$\frac{p_1}{p_2} = \left(\frac{E_{\text{H}_2\text{O}}}{E_{\text{O}_2}}\right)^2 \times \frac{S_2 \Delta\sigma_2}{S_1 \Delta\sigma_1} \quad (1)$$

where p_1 , p_2 are the mean pressures of water vapour and oxygen (atm); S_1 , S_2 are the line strengths of the two lines (cm⁻² atm⁻¹); and $\Delta\sigma_1$, $\Delta\sigma_2$ are the line widths of the two lines (cm⁻¹). Now the mass mixing ratio μ is related to equation 1 by

$$\mu = \frac{p_1}{p_2} \times \frac{M_1}{M_2} \times \frac{k_2}{k_0} \quad (2)$$

where M_1 and M_2 are the molecular weights of water vapour and oxygen; and k_2 and k_0 are the mean con-

centrations of oxygen and air in g m^{-3} over the emission path; so that, in units of g/g ,

$$\mu = \left(\frac{E_{\text{H}_2\text{O}}}{E_{\text{O}_2}} \right)^2 \times \frac{S_2 \Delta\sigma_2}{S_1 \Delta\sigma_1} \times \frac{M_1 k_2}{M_2 k_0} \quad (3)$$

Thus in order to derive μ only $[E_{\text{H}_2\text{O}}/E_{\text{O}_2}]^2$ has to be measured experimentally, the other terms either being well established (M_1 , M_2 , k_2 and k_0) or theoretically evaluated for specific lines (S_1 , S_2 , $\Delta\sigma_1$ and $\Delta\sigma_2$) (refs. 11–14). In the case of line parameters, experimental work in the millimetre and submillimetre region has provided support for the theoretical calculations and shown that systematic errors in theory of $\Delta\sigma_1$ and $\Delta\sigma_2$ tend to be reduced using the ratio $(\Delta\sigma_1/\Delta\sigma_2)$ (refs. 15–18). Using this technique, a value of $\mu = 2.0 \times 10^{-6} \text{ g/g}$ was obtained for the pair of lines shown in Fig. 2, and a mean value for all the lines accessible in the range 20 to 65 cm^{-1} (that is, clearly resolved pairs of oxygen and water vapour lines of comparable intensity and not affected by other atmospheric constituents such as ozone) was

$$\mu = (2.1 \pm 0.3) \times 10^{-6} \text{ g/g}$$

The quoted errors are the r.m.s. deviation of the values of μ measured, and thus provide a measure of the possible accuracy of the experimental technique, but no allowance has been made for the systematic errors that may be present either in the line parameters or in the analysis. This value of the stratospheric mixing ratio is in good agreement with other results for the dry stratosphere model^{1,2}. The effect of water vapour in the optical path (about 1 m) in the aircraft was less than 5 per cent of emission from the atmosphere and can thus be considered negligible in this experiment.

This result applies to the lower stratosphere only; the energy of emission in the "strong" lines used decreases away from the observer, falling to $1/e$ in a distance corresponding to a vertical height of about 3 or 4 km above the aircraft. The measurement of weak emission lines will yield information about higher altitudes, and these are being investigated in the more detailed analysis of the present results to be published later.

The value of this method of measuring the stratospheric mixing ratio μ may be summarized by considering the following points.

(a) The relative strengths of the strong electric dipole transitions of water vapour (approximately $2 \text{ cm}^{-2} \text{ atm}^{-1}$) and the weak magnetic dipole transitions of oxygen (approximately $1 \times 10^{-5} \text{ cm}^{-2} \text{ atm}^{-1}$) are particularly suited to measuring water vapour to air mixing ratios in the range 10^{-6} to 10^{-5} g/g , which are encountered in the stratosphere.

(b) The existence of a considerable number of oxygen transitions in the submillimetre region which can be resolved from nearby water vapour lines enables increased accuracy to be obtained by measuring several sets of features. Further, the multiplicity of both strong and weak water vapour lines which are available for consideration will probably allow an extension of the analysis to include more complex distributions of water vapour in the stratosphere².

(c) In balloon work, though not of such importance in aircraft work (see above), the use of atmospheric emission enables long path lengths to be considered, and consequently reduces the problem of local contamination caused by water vapour outgassing from the apparatus.

Finally, the combination of the inherent advantages of Fourier transform spectrometry in the submillimetre region, the more complete information of the parameters of the rotation lines of water vapour and oxygen, and the elimination of the need for guidance equipment (a requirement for comparable solar studies) in atmospheric emission measurements seem to recommend this technique strongly for future studies of the mixing ratio in the stratosphere.

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The Classical Charged Photon

BONNOR^{1,2} has given a solution of Maxwell's equations which can be interpreted as a radiative field caused by a source moving at the speed of light. Because the world-line of the source is therefore a null curve Γ , the current four-vector J^i is everywhere tangent to it. Hence

$$g_{ik} J^i J^k = 0 \quad (1)$$

where g_{ik} is the metric tensor in local coordinates. In Bonnor's solution L^i is always parallel to J^i , that is

$$J^i = Q L^i \quad (2)$$

where Q is a scalar field. It will be shown below that in Minkowski space, for a null (or radiative) solution of Maxwell's equations satisfying equations (1) and (2) only, the radiation is propagated along a family of null straight lines which is, in general, shear-free, expanding and twisting. Further, in the absence of twisting, Q in these solutions vanished at every point of Γ . Bonnor's rather strange solution arises as the singular solution of equation (10) below, which gives zero expansion and twist.

Now, radiation problems in space-times can be discussed very conveniently in terms of tetrads of null-vectors (L^i , N^i , M^i , \bar{M}^i), where L^i and N^i are real, M^i and \bar{M}^i are complex conjugates

$$\begin{aligned} L^i L_i = 0, \quad N^i N_i = 0, \quad M^i M_i = 0, \quad \bar{M}^i \bar{M}_i = 0, \\ L^i N_i = 1, \quad M^i \bar{M}_i = -1 \end{aligned} \quad (3)$$

and all remaining scalar products vanish³. (I write $\pi = -N_{ik} \bar{M}^i L^k$ to avoid confusion with the transcendental real number π .) Instead of coordinate derivatives $\partial/\partial x^i$ and Christoffel symbols it is more convenient to use the linear differential operators

$$D = L^i \frac{\partial}{\partial x^i}, \quad \Delta = N^i \frac{\partial}{\partial x^i}, \quad \delta = M^i \frac{\partial}{\partial x^i}, \quad \bar{\delta} = \bar{M}^i \frac{\partial}{\partial x^i} \quad (4)$$

and the Newman-Penrose spin coefficients $\alpha, \beta, \gamma, \varepsilon, \kappa, \lambda, \mu, \nu, \pi, \rho, \sigma, \tau$ respectively³. The Maxwell equations with source

$$J^i = i^1 L^i + i^2 N^i + i^3 \bar{M}^i + i^4 \bar{M}^i \quad (5)$$

become

$$\begin{aligned} D\varphi_i - \bar{\delta}\varphi_0 &= (\pi - 2\alpha)\varphi_0 + 2\rho\varphi_1 - \kappa\varphi_2 + \frac{2\pi}{c}i^2 \\ D\varphi_2 - \bar{\delta}\varphi_1 &= -\lambda\varphi_0 + 2\pi\varphi_1 + (\rho - 2\varepsilon)\varphi_2 - \frac{2\pi}{c}i^3 \\ \delta\varphi_1 - \Delta\varphi_0 &= (\mu - 2\gamma)\varphi_0 + 2\tau\varphi_1 - \sigma\varphi_2 + \frac{2\pi}{c}i^4 \\ \delta\varphi_2 - \Delta\varphi_1 &= -\nu\varphi_0 + 2\mu\varphi_1 + (\tau - 2\beta)\varphi_2 + \frac{2\pi}{c}i^1 \end{aligned} \quad (6)$$

where $\varphi_0 = F_{ik}L^i\bar{M}^k$, $\varphi_1 = \frac{1}{2}F_{ik}(L^iN^k + \bar{M}^i\bar{M}^k)$, $\varphi_2 = F_{ik}\bar{M}^iN^k$ are the complex field strengths³. For a null field we can always choose the tetrad⁴ such that

$$\varphi_0 = \varphi_1 = 0, \varphi_2 \neq 0 \quad (7)$$

To make equations (2) and (5) consistent we must have

$$i^1 \neq 0, i^2 = i^3 = i^4 = 0 \quad (8)$$

Substituting (7) and (8) in (5) gives $\kappa = \sigma = 0$ for the L^i -congruence, which means that the radiation field propagates along shear-free null geodesics³.

From the conservation of charge equation $J^i{}_{;i} = 0$ it follows that on

$$\begin{aligned} Q, iL^i + QL^i{}_{;i} &= 0 \\ DQ &= -QL^i{}_{;i} \\ &= Q(\rho + \bar{\rho}) \end{aligned} \quad (9)$$

By a change of scale along L^i we can make $\varepsilon + \bar{\varepsilon}$ vanish and hence get the simple differential equation for ρ in Minkowski space

$$D\rho = \rho^2 \quad (10)$$

It is well known^{5,6} that an affine parameter r can be found on each curve of the L^i -congruence such that $D = \partial/\partial r$. This gives for equation (10) the general solution

$$\rho = (r + a + ib)^{-1} \quad (11)$$

where a and b are independent of r . The singular solution $\rho = 0$ giving zero expansion and zero twist is Bonnor's. From equation (9), Q must be constant in this situation. Taking $a = 0$ merely changes the origin of r on each curve but does not alter the problem. Thus equation (9) becomes on Γ

$$DQ = 2Qr(r^2 + b^2)^{-1} \quad (12)$$

which has the general solution

$$Q = K(r^2 + b^2) \quad (13)$$

where K is also independent of r . Clearly when $b = 0$ we see that Q must vanish at $r = 0$ and hence by affine transformations at every other point of the geodesic Γ .

Finally,

$$\begin{aligned} \rho &= 0 + i\omega \\ &= \frac{r}{r^2 + b^2} + i \frac{(-b)}{r^2 + b^2} \end{aligned} \quad (14)$$

showing that when $b \neq 0$ the L^i -congruence has non-zero local expansion θ and twist ω (refs. 2, 5 and 6).

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Results of a Seismic Refraction Experiment on Rockall Bank

ROCKALL BANK (Fig. 1) is situated in the NE Atlantic 300 miles west of Scotland and represents the shallowest part of Rockall Plateau, a more extensive shoal area. The Plateau is less than 1,000 fathoms in depth and the Bank is less than 500 fathoms, culminating in Rockall Islet at its north-east end. Rockall Trough, 1,500 fathoms deep, separates Rockall Plateau from the continental shelf west of Scotland.

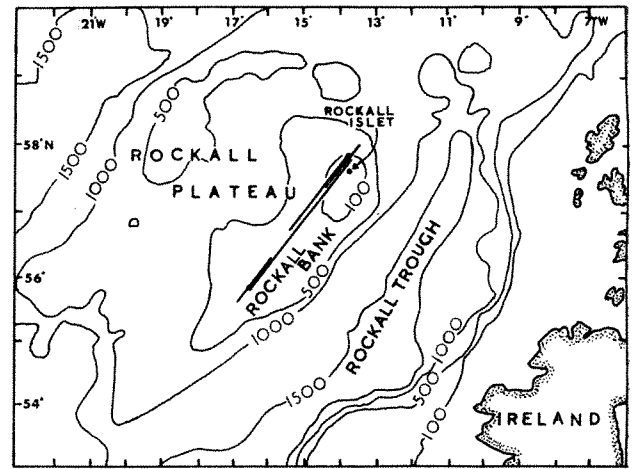


Fig. 1. The location of the seismic refraction experiment on Rockall Bank. The lines of shots and positions of receivers are shown.

A 150 nautical miles long reversed seismic refraction line, incorporating short split profiles at each end, was shot along Rockall Bank from RRS Discovery in the autumn of 1969 in order to obtain a thickness for the Earth's crust beneath the Bank. The internally recording sono-radio buoys of Cambridge University and the pop-up bottom siesmometers (PUBS) of the National Institute of Oceanography acted as receivers while Discovery steamed away firing charges up to 900 pounds at 150 nm.

About two hundred points from the sono-buoy data were used to construct a travel-time plot for the split and reversed profiles (Fig. 2). A plot of about forty points from the PUBS data, not included in Fig. 2, confirms the sono-buoy results. An offset of 0.4 s occurs in the 6.41 km/s line at 35 s from the north-east end of the profile. It can be attributed to a structural step in the refracting interface (Fig. 3b). Because of bad weather the refraction line was not completely reversed, so a velocity for the upper mantle was obtained in one direction only, being 8.2 km/s. The formulae of Ewing *et al.*¹ were used to calculate the velocities, depths and dips of the refracting layers of the crust. The data from the split profiles were used to calculate the shallow structure at each end of the line (Fig. 3a) and the data from the reversed part of the line were used to calculate the deep structure of the Bank (Fig. 3b).

There is no evidence for superficial unconsolidated sediment cover to Rockall Bank at either end of the line. Seismic reflexion profiling records over the Bank show that superficial sediments occur only as a thin layer and in pockets². The 3.8 km/s velocity measured at the north-east

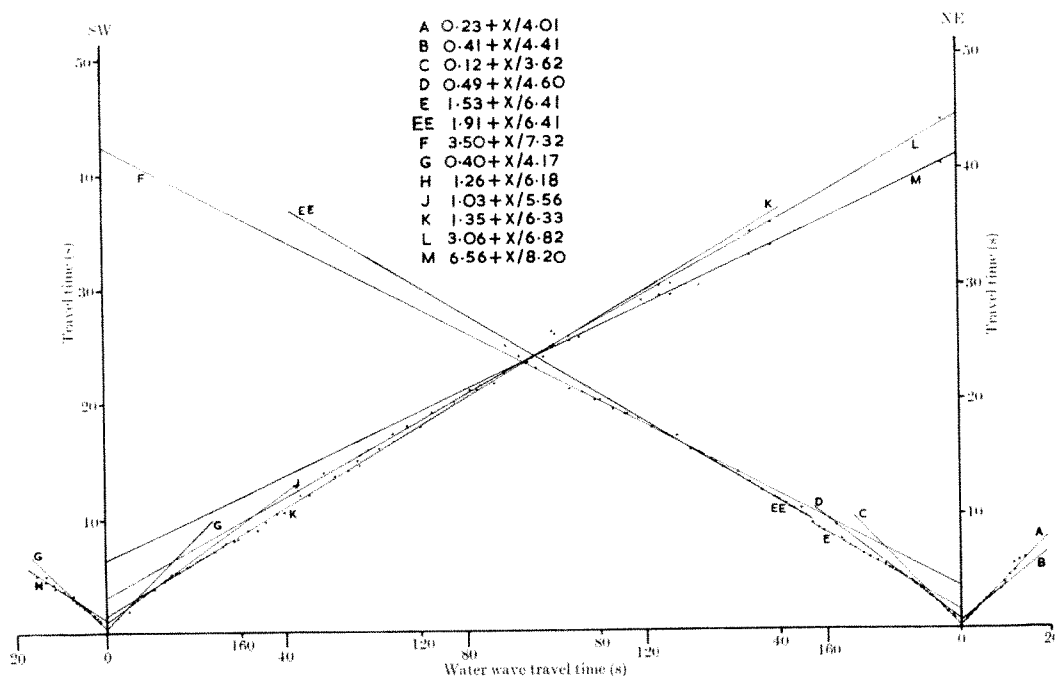


Fig. 2. Travel-time plot for the seismic refraction experiment on Rockall Bank. Equations to the lines are expressed in the form: intercept time (s) + X /velocity (km/s).

end of the line is close to the 3.9 km/s obtained for basalts in the Faroe Islands and Iceland². Basalts have been dredged from Rockall Bank^{4,5} and Rockall Islet is probably a remnant of a Tertiary volcanic centre⁶⁻⁸. This evidence suggests that the 3.8 km/s layer may represent basaltic lava flows on Rockall Bank, perhaps as part of the Thulean volcanic province⁴. The 5.85 km/s velocity found only at the south-west end of the Bank is within the range of velocities assigned to the granitic layer of the crust^{9,10}, but here it may well represent very old sedimentary rocks. The shallow structure is, no doubt, highly variable over the

length of the refraction line, so to calculate the deep structure a mean velocity of 4.8 km/s was used for the top crustal layer. The 6.36 km/s velocity obtained for the upper crust is well determined, but the 7.02 km/s velocity for the lower crust is less well determined. Both these velocities are at the upper limit of those quoted for a typical continental crust^{9,10}. The interface at 15 km depth appears to be a moderately strong refractor on the evidence from the travel-time plot, and can be identified with the Conrad discontinuity. The most important result of the whole experiment, however, is the value of approximately 31 km for the depth to the Moho beneath Rockall Bank.

This direct evidence for the continental thickness of the Earth's crust beneath Rockall Bank, together with the evidence from compiled gravity and magnetic data, justifies the retention of Rockall Bank in the pre-drift reconstruction by Bullard *et al.*¹¹ of the North Atlantic continents.

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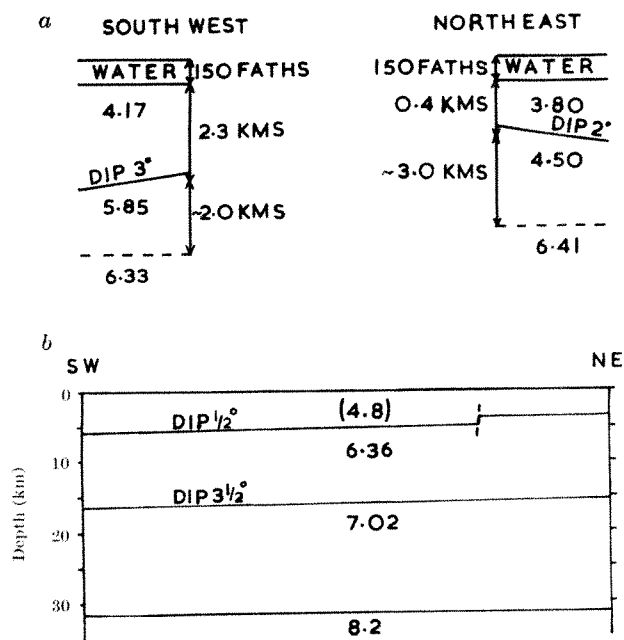


Fig. 3. *a*, Shallow structure of Rockall Bank (not to scale). *b*, Deep structure of Rockall Bank. Vertical exaggeration 4:1. Velocities in km/s; (4.8) mean velocity.

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Origin of the Hurd Deep, English Channel

THE Hurd Deep, the largest deep in the English Channel, is a linear depression about 95 miles (150 km) long, 1-3 miles (1.5-5 km) wide and about 30-50 fathoms (55-90 m) deeper than the adjacent sea floor. The depression follows a relatively straight course WSW from near the north-west corner of Cherbourg Peninsula and runs parallel to the general structural trend of the western English Channel.

sions which form a tributary system. These depressions are partially filled channels, and they can be traced eastwards towards the Seine, Somme and Straits of Dover¹².

The Hurd Deep is the remnant of a much larger channel system, now partially filled in. We conclude that the erosion of the channel now forming the Hurd Deep was caused by both fluvial and tidal action. Fluvial erosion was related to downcutting by rivers extended across the continental platform during periods of low Pleistocene sea-level. During this time, the lower reaches of the river system would have been subjected to strong scouring by

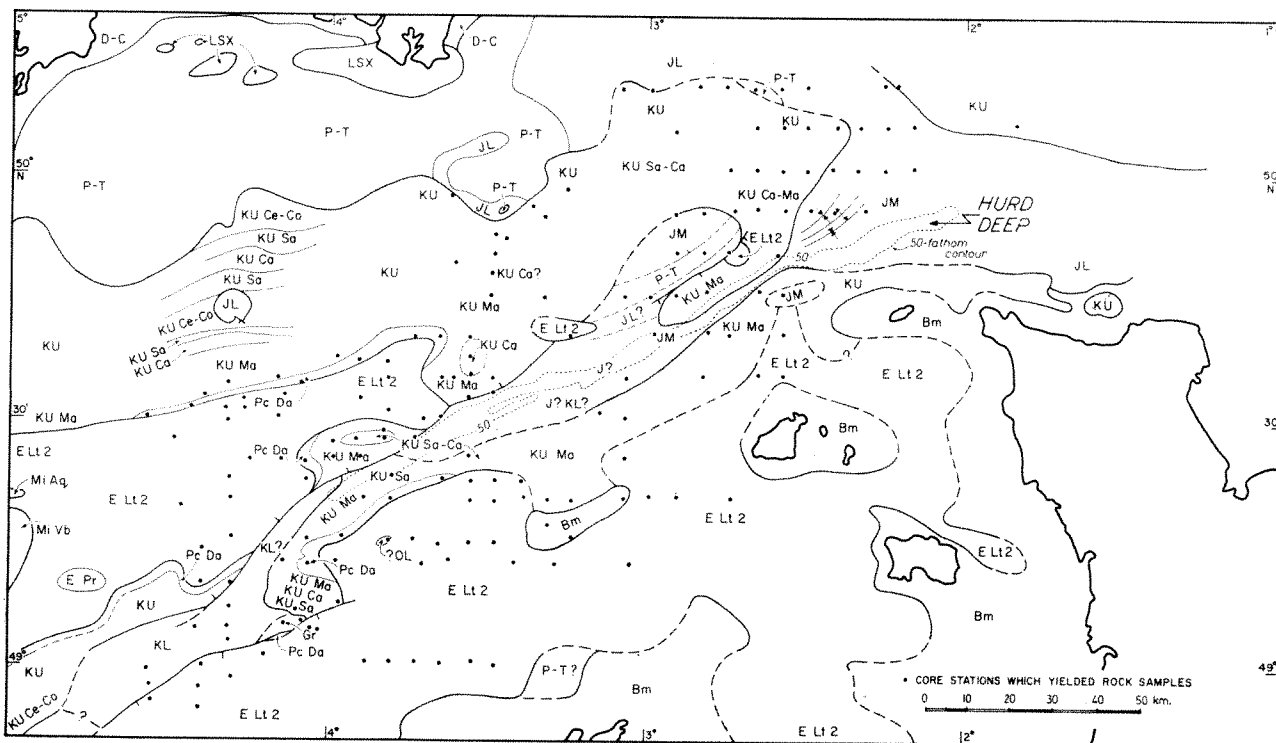


Fig. 1. Geological map of the Hurd Deep area, English Channel. Bm, Basement; LSX, Lizard-Start complex; Gr, Granite; D-C, Devonian Carboniferous; P-T, Permian-Triassic; JL, Lower Jurassic; JM, Middle Jurassic; KL, Lower Cretaceous; KU Ce, Upper Cretaceous, Cenomanian; KU Co, Coniacian; KU Sa, Santonian; KU Ca, Campanian; KU Ma, Maestrichtian; Pc Da, Palaeocene Danian; E Pr, Eocene, Priabonian; E Lt2, Eocene, Upper Lutetian; Ol, Oligocene; Mi Aq, Miocene Aquitanian; Mi Vb, Miocene Vindobonian.

Detailed geological mapping of the area by the Bristol University Group has brought to light new data which permit a critical assessment of the numerous hypotheses that have been put forward to account for the origin of the deep. These hypotheses suggest river erosion¹⁻³, faulting and structural control⁴⁻⁶, glacial erosion^{7,8}, a karstic origin in chalk⁹ and tidal erosion^{3,10}.

Using a combination of gravity coring and sparker surveys, the Bristol University Group has produced a detailed map of the solid geology of the Western Approaches to the English Channel¹¹. The part of this geological map in the region of the Hurd Deep shown as Fig. 1 is based on 52 sparker traverses and some 200 gravity core stations.

The map clearly indicates an erosional origin. For about 60 miles (95 km) erosion has cut through chalk sediments into the underlying Jurassic rocks. The fact that for over 80 miles (125 km) the Deep is floored by Jurassic clays, silts and thin limestones renders the hypothesis of karstic origin⁹ untenable.

Further support for an erosional origin comes from the sparker profiles which reveal that the Hurd Deep is a partially filled channel. The depth of fill in the channel is variable, reaching 200-300 feet (60-90 m) in some places and decreasing to zero at the south-western end of the feature. At the north-eastern end there are three depres-

sions which form a tributary system. As sea-level rose, this latter mechanism has, no doubt, been a major factor in maintaining the deep up to the present day, because the maximum depths in the feature now occur in the region of the strongest tidal currents¹⁰.

We thank the director, Institute of Geological Sciences, London, for permission to reproduce Fig. 1.

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Himalayan Ridge in the Light of the Theory of Continental Drift

A UNIQUE feature of the geology of the Himalayas is the presence to the north of the Great Himalayan Range of a complete sequence of marine fossiliferous rocks ranging in age from the Cambrian to Eocene and an enigmatic absence of fossils in the pre-Tertiary sediments lying to the south of the range¹. A notable exception is found in Kashmir where fossiliferous Cambrian to Jurassic marine rocks lie to the south of the Great Himalayan Range as a result of the Kashmir Nappe². Geologists working in the Himalayas have tried to explain this anomaly by erecting a hypothetical barrier between the northern and southern basins to prevent the migration of animals and plants from north to south. This barrier was called by Wadia³ a "geanticline within the main geosyncline". Pande and Saxena⁴ thought that the geanticline or central barrier was formed during the Ordovician times. Fuchs⁵ named this imaginary barrier "Himalayan Ridge" and thought that it was formed after the Caledonian orogeny. It has also been suggested that this geanticline or ridge has been subjected to denudational processes since its origin in the early Palaeozoic times, but it has become the highest mountain range in the world and it is still rising.

Recent palaeomagnetic studies on the Deccan Plateau basalts have revealed that the Indian Peninsula has drifted about 5,000 km in a northerly direction since the end of the Cretaceous at a rate of about 7 cm a year⁶. The Indian Peninsula probably lays further to the south in the pre-Cretaceous times. This important discovery has given strong support to Wegener's theory of continental drift⁷. Geologists working in the Himalayas, however, seem to have overlooked the great implications which follow from the above discovery. We should like to point out that if the Indian Peninsula along with the sediments laid down on its northern margin and which now constitute the Lesser Himalayan zone lay at least 5,000 km to the south of its present position in late Cretaceous, then the presence of a geanticline or ridge becomes superfluous. The above contention is supported by the fact that the Himalayas have a maximum width of 400 km. Gansser⁸ suggested a maximum shortening of the crust of the order of 500 km during the Himalayan orogeny. This would mean a maximum width of only 900 km for the so-called northern and southern geosynclines. What, then, happened to the remaining 4,000 km of land or sea or both?

In the light of the above facts we suggest that the Tethyan zone lying to the north of the Great Himalayan and the Lesser Himalayan zone lying to the south of the range were at least 5,000 km apart. This would explain faunal differences of the two and obviate the necessity of having a ridge in pre-Tertiary times.

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Evidence for Salt Deposits in the Appalachian/Caledonian Orogen

ALBITE-oligoclase feldspar concentrations occur abundantly in the Eocambrian to Lower Ordovician metasediments, along the entire metamorphic Caledonian-Appalachian mountain chain. In Spitsbergen (refs. 1 and 2 and personal communication from W. Horsfield); Stavanger, Norway; SE Scotland; Antrim, N. Ireland; Co. Mayo, Ireland³; Burlington Peninsula, Newfoundland; Pennsylvania; Dutchess County, New York⁴; and Maryland, particular attention has been given to the albite schist association.

The origin of the large amounts of Na₂O involved in these albite schists is of great importance. It is usually suggested that regional soda metasomatism, usually involving fluids from granites, soda granites and their associated migmatites, is responsible and sedimentary or even volcanic origins have also been suggested. A completely satisfactory explanation for these enormous quantities of Na₂O has, however, not yet been given.

The albite schists occur in different parts of the sedimentary pile, and they are associated with similar lithologies. Volcanic rocks are common, but are not obvious in all cases. In most areas, the albite schists are stratigraphically and lithologically controlled and a primary origin of the sediments and element distribution can be demonstrated. The regional metasomatic origin, which is preferred in many cases only because a primary origin for the amounts of Na₂O and K₂O could not be found, therefore seems unreasonable.

Thick salt layers are often associated with subsiding rifts, in which the salt and clastics directly overlie a volcanic basement. In the Red Sea, Miocene basal clastic sediments are overlain by gypsum, probably of continental origin. Upper Miocene sediments are brackish to marine, and by the Pliocene, marine sedimentation was widespread. Large thick evaporite and associated metal concentrations are known to exist in the sediment piles up to 15 km thick. Of very great interest are the hot brine pools found in the axial rift of the Red Sea⁶. Their composition strongly supports the evidence that they are not a single concentrate developed by extreme evaporation, and suggests that they are a resolution of previously deposited halite and gypsum deposits together with their trace elements, long since parted from their bittern, which remained in solution and were possibly deposited elsewhere. It is clear that the chemistry of the present hot brines involves numerous complex chemical reactions and inter-relationships. Element enrichment is comparable with that of salt in salt domes of non-volcanic areas.

Further south, along the rift valleys of East Africa, extensive continental deposits of limited thickness contain large amounts of saline evaporites, zeolites, authigenic sodium feldspars and potassium (ref. 7 and personal communication from J. A. Van Couvering). These are partly a direct consequence of the associated volcanics along the fault scarps.

Around the margins of the present Atlantic Ocean, salt is widespread. Association of extensive salt doming with oil and gas is common⁷ and evaporite basins are common along the African coast (Fig. 1). It has been suggested⁸ that salt deposition is genetically related to the initial stage of rifting and was widespread during the initial opening of the present Atlantic Ocean, in certain latitudes. Elsewhere, such as in the western Mediterranean, segments of ocean floor formed during the Tertiary are now extensively covered by clastic sediments which include known salt deposits⁸.

In a general model describing the opening of a new ocean basin⁹ in terms of plate accretion, an integrated relationship between graben formation, rapid sedimentation and seafloor spreading are envisaged. The situation can be related rather well to the present situations in the

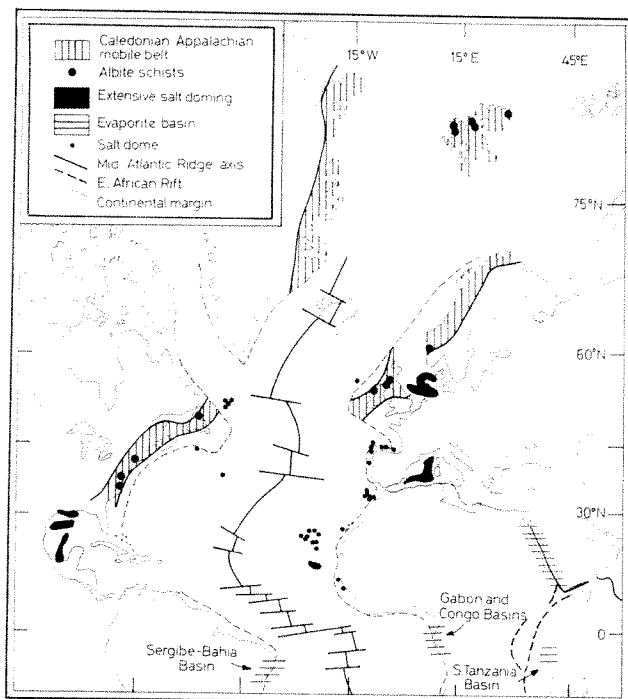


Fig. 1. Albite-schist occurrences in the Appalachian/Caledonian Orogen and salt occurrences in the Atlantic Ocean.

African rift valley and the Red Sea, and at a later stage to the present Atlantic ocean.

It has been suggested that a proto-Atlantic ocean¹⁰ opened and closed along the Caledonian-Appalachian suture¹¹ in a complex manner in terms of plate tectonics¹². Marginal sedimentation along the proto-Atlantic continental margins was recognizably very similar along the entire site. Rapid deposition of coarse shallow water clastics are followed by extensive quartzites, carbonates, volcanics and graywackes.

Evaporites are suggested to have been a major early component of these thick sediment wedges along the proto-Atlantic continental margin. This is indicated by extensive halite pseudomorphs in Greenland¹³, but it must be realized that the evolution of salt deposits, subsequent to deposition, can be extremely complex¹⁴. Minerals may be remobilized and widely disseminated, or concentrated in layers throughout the sediment pile. The premetamorphic evaporites almost certainly underwent complex authigenic growth, brine distribution and redistribution, normal geothermal metamorphism, and complex salt tectonics, under the sedimentary overburden during prolonged diagenesis, and this could explain the mainly stratigraphic variations. The numerous possibilities of remobilization of the salt components during orogeny may be even more complex and this may be why extensive evaporites have so far not been described in metamorphic terrains. That they exist, however, has been shown by the presence of gypsum, halite, graphites and paraffins in areas such as the Grenville belt¹⁵.

The evaporites and related sediments (personal communication from J. F. Dewey) may remain on a descending oceanic plate, the Arabian Shield evaporites. Or, in conditions of orthotectonic deformation and metamorphism, the salt components may undergo complex reactions with co-existing silicates in the elevated P and T conditions. This leads to the extensive development of albite oligoclase schists in large areas of the Caledonian-Appalachian belt, and to the sometimes widespread developments of scapolite^{16,17}.

The distribution after metamorphism is still essentially stratigraphically and lithologically controlled, which

agrees well with the postulated sedimentary origins of the soda.

This may thus reflect a much greater relationship in some areas between mineral assemblages and the original chemical composition than has previously been thought. Certainly, the large scale regional metasomatic processes so often envisaged in this context need not necessarily have occurred.

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Voids in Nickel irradiated with Electrons after Previous Argon Ion Bombardment

VOIDS can be produced in some metals by high fluence neutron irradiation at temperatures between $0.3 T_m$ and $0.5 T_m$, where T_m is the melting temperature. The effect was found first in stainless steel¹ and subsequently in several pure metals including nickel^{2,3}. Void formation

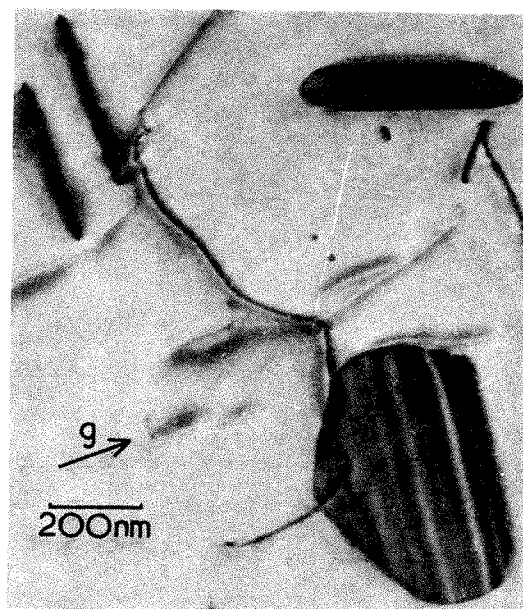


Fig. 1. Large faulted loops in nickel, electron irradiated at 430° C without previous ion bombardment; foil normal (110), $g = (002)$.

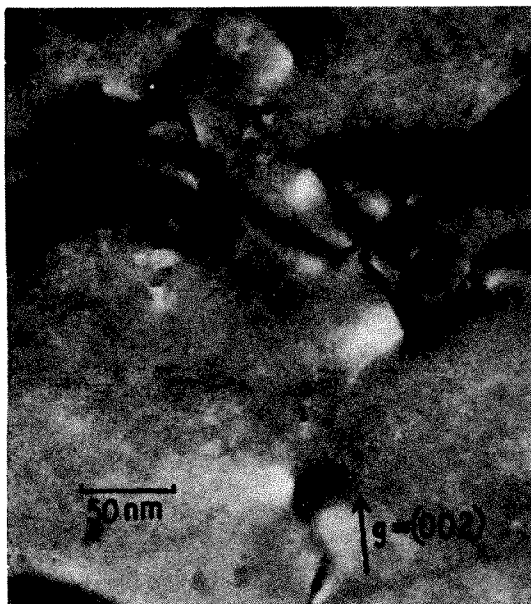


Fig. 2. Voids and dislocations in nickel given the identical electron irradiation to that of Fig. 1 but after argon ion bombardment.

causes dimensional instability and is liable to be a serious technological problem in fast reactor fuel cladding. This note reports some preliminary results on the production of voids in nickel by electron irradiation in the beam of a high voltage electron microscope (HVEM) following an initial argon ion bombardment. The HVEM offers a convenient method of studying electron displacement damage effects⁴ and in this work we used a Hitachi 1 MV instrument with a heating stage.

Vacuum annealed nickel sheet of 99.995 per cent purity was electro-polished to form thin foil specimens, some of which were then bombarded with 130 kV argon ions to various fluences before insertion in the HVEM. Electron bombardment was at 1 MV with a beam current of 0.4 μA (measured below the specimen) and with a beam diameter $\sim 3 \mu\text{m}$. A 1 MV electron can transfer to a nickel atom a maximum of 70 eV, sufficient to displace two atoms at most. If we take the displacement cross-section as 34 b, the fraction of atoms displaced per second is 1.6×10^{-3} . All nickel specimens placed in the HVEM beam are observed at first to develop black spots which then grow and interact. In a specimen not previously ion bombarded, electron irradiation at 430° C results in the growth of very large faulted loops (see Fig. 1), similar to those previously observed in copper irradiated with electrons at an unknown temperature⁵. An ion bombarded specimen contains initial black spot damage but no features identifiable as voids. As exposure to the HVEM beam continues, a totally different electron irradiation damage structure is produced. Fig. 2 shows a nickel specimen bombarded with 130 kV argon ions to a fluence of $2 \times 10^{18} \text{ m}^{-2}$ and then irradiated in the HVEM beam for 40 min at 430° C. A network of dislocation tangles and loops has been produced, together with a high density of small, approximately square features, some darker and some lighter than background. These have the characteristics of diffraction contrast from small voids⁶; the contrast may be positive or negative depending on the excitation error and on the vertical position of the void. The features are therefore identified as voids within the foil. The void density is $2 \times 10^{15} \text{ m}^{-2}$ for a foil thickness of 130 nm. The images have edges along the projected (100) directions and the average edge length is 6 nm. In some cases voids lie along a line, possibly that of a dislocation which has subsequently moved.

Calculations were performed to determine whether the features could be equilibrium bubbles resulting from the argon ion bombardment. Taking the surface energy to be 1.6 J m^{-2} , the observed features would require 8×10^{18} gas atoms m^{-2} , a factor of 4 greater than the actual argon ion fluence. Further, a specimen ion bombarded to a fluence 100 times greater gave features of similar size and density. Consequently the conclusion is that the features cannot be equilibrium bubbles and must be regarded as voids.

This is the first observation of void formation by electron irradiation. The voids were only found in pre-bombarded specimens indicates the importance of either or both gas atoms and point defect clusters on the nucleation process. The result shows that void growth occurs during irradiation which produces only mono-vacancies or di-vacancies but no larger cascades. Irradiation in the beam of a HVEM offers an excellent method of studying the formation and properties of voids. The effects of varying the pre-bombardment conditions and the HVEM beam irradiation conditions are being investigated in detail and will be reported elsewhere.

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Overexcitation Interferences in Atomic Absorption Spectrophotometry with an Air-Acetylene Flame

In recent years, atomic absorption spectrophotometry has established itself as a valuable analytical technique for trace metal analysis and a large number of procedures are now available for the determination of metals in a wide variety of matrices^{1,2}. In most cases, only qualitative interference effects have been considered, and very little research appears to have been directed towards the flame chemistry of the technique. This would help to elucidate the mechanism of metal vapour production and the causes or mechanisms by which flame interferences are produced. The only interference phenomena which are now well established are those relating to ionization and to the formation of thermally stable compounds such as single or mixed oxides.

We have recently been examining interference effects of transition metals and have noted the anomalous effect of flame mixture ratio (air : acetylene) on the absorption of several metals. Solutions of metal salts, as sulphates or nitrates, which would be expected to yield a metal oxide (MO) as the penultimate species before the formation of free metal atoms, exhibit a sharp drop in absorbance occurring at a particular mixture ratio and all mixtures containing more acetylene on the fuel-rich side of the flame. In Fig. 1 the absorption due to iron at various flame mixture compositions is illustrated and shows a sharp drop at acetylene : air ratios greater than 0.85. These effects are not observed in chloride medium and only occur under conditions which would be expected to favour oxide dissociation (that is fuel rich) and in fact the variation in emission intensity of the FeO band (measured at 574 nm) decreases with increasing acetylene concentration, which supports this hypothesis.

Our results to date indicate that this phenomenon is most prevalent for those elements exhibiting complex atomic spectra in which there are possibly long lived metastable states of higher energy than the ground state. It seems likely that the loss of absorption in a fuel-rich air-acetylene flame may be caused by the production of metal atoms in some of these states rather than in the ground state and that some of the interferences which have been reported^{3,4} to occur in these conditions (that is rich flame, sulphate medium) may also be attributed to the same phenomena.

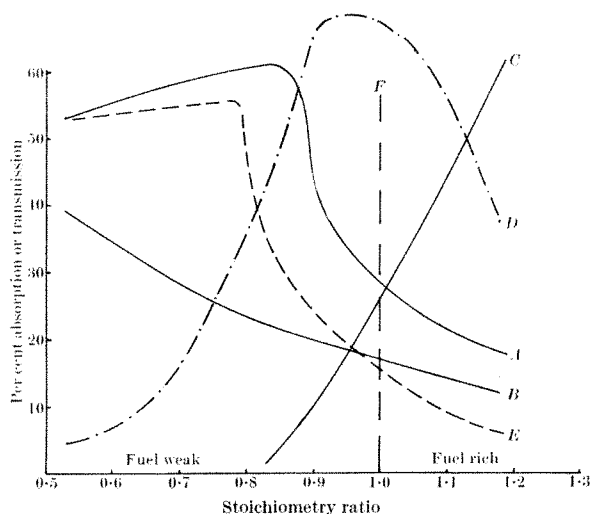


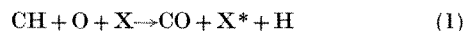
Fig. 1. Variation of the absorption and emission of several species with respect to flame composition. Curve A, 25 p.p.m. iron (III) sulphate in 10^{-3} M sulphuric acid (absorption). Curve B, emission due to FeO, produced from a solution of 1,000 p.p.m. iron as iron (III) sulphate. Curve C, emission due to C_2 . Curve D, 25 p.p.m. chromium (III) sulphate in 10^{-3} M sulphuric acid (absorption). Curve E, 25 p.p.m. cobalt (II) sulphate in 10^{-3} M sulphuric acid (absorption). The position of stoichiometric composition is indicated at F.

The absorption profiles (with respect to fuel-air ratio) of Fe, Cr, Ni, Co, Cu, Ru, Mn, Ca, Mg, Zn in both sulphate and chloride media were recorded using a standard Perkin Elmer 290 Atomic Absorption Spectrophotometer with an air flow rate held constant at 46.1 min^{-1} (corrected to atmospheric pressure) and acetylene flow rate varied between 2.0 and 4.8 l min^{-1} . Standard Perkin-Elmer hollow cathode lamps were used in each case and operated at the recommended current and wavelength settings. Percentage absorption measurements were observed with the centre of the light beam at 10 mm above the top of the standard 50 mm slot burner. Emission data were obtained using the manufacturer's emission accessory with the flame placed at 90° to the light path and masked to give a 2 mm deep horizontal slit centred at 10 mm above the burner head.

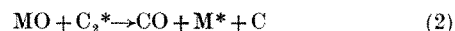
The sharp drop in absorption due to iron, chromium and cobalt at all acetylene to air ratios greater than the particular critical ratio, which varies from element to element, is shown in Fig. 1, and we have found similar behaviour with Ni and to a lesser extent with Cu. The effect is, however, absent for Mn and Zn. The effect may also be observed at similar mixture ratios in air-propane flame but is absent from the air-hydrogen flame in which carbon species are not present. Severe interferences have been reported^{3,4} for iron in these fuel-rich conditions and similar interferences are observed with Co, Ni and Cr.

The mixture ratios at which these effects are observed coincide with the region in which emission from carbon species such as C_2 , CH and CO are found to persist at higher parts of the flame where absorbance measurements are normally made (as distinct from the primary cone where such emission may be observed in all flame conditions).

The variation of C_2 emission intensity with flame gas mixture measured at 517 nm is shown in Fig. 1, and the CH profile measured at 430 nm is very similar. Over-excited C_2 or CH species, among others, are known to occur in these conditions⁵, and arise from reactions such as



where X is an energy acceptor (for example, another C_2 or CH molecule) which can accept the 176 kcal of energy liberated in this reaction. Such overexcitation is most strongly observed in an air-acetylene flame and does not occur to any significant extent in hydrogen flames where the flame reactions are less exothermic⁵. Over-excitation of metal atoms has been confirmed for several metals⁶⁻⁷ and may occur through reaction (1), where X is the metal atom, by direct overexcitation energy transfer or most likely, through the reaction of the metal oxide species with an overexcited molecule, for example C_2^* ,



It would be most prevalent in conditions in which the concentration of oxygen is low and fails to react with all the C_2 produced, in a fuel-rich air-acetylene flame.

Additional evidence for this hypothesis is to be presented elsewhere, but these effects illustrate the importance of careful control of operating conditions with respect to both flame parameters and the chemical composition of the samples and standard solutions used in atomic absorption spectrophotometry, even for elements previously considered to be relatively interference free.

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Homogeneous Photocatalysis by Organic Dyes in the Liquid Phase

EXPERIMENTS using organic semiconductors as catalysts are interesting for several reasons. Organic semiconductors have a molecular lattice and the parameters of the catalyst can therefore be altered by altering the molecules forming the lattice. Organic chemistry techniques also enable single molecules to be synthesized in a tailor made fashion.

We have studied various heterogeneous reactions catalysed in dark and light by organic semiconductor crystal powders in experiments in a static apparatus. These reactions are oxidation of carbon monoxide photocatalysed by crystal violet, metal free and Cu-phthalocyanine, the oxidation of H_2 to H_2O and the decomposition of N_2O photocatalysed both by metal free and Cu-phthalocyanine. Together with the catalytic reaction, a

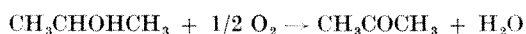
more or less pronounced decomposition or oxidation of the organic catalyst occurred, and we have tried to suppress this by varying the reaction conditions and the experimental techniques. The decomposition of formic acid and of isopropyl alcohol with *p*-bromanil and *p*-chloranil as catalysts has been established by gas chromatography, without any disturbance. These reactions are now being studied in more detail.

In reactions catalysed by crystalline dyes, are the crystal properties or the structure of the single surface molecule of the dye responsible for the catalytic processes observed? Furthermore, because the dye molecule is rather large in comparison with the substrate molecule, the interaction of the substrate molecule with the crystal surface may involve only one surface molecule of the dye. This interaction would certainly be influenced by the interaction of the surface dye molecule with the lattice of the dye. It is therefore necessary to carry out experiments which can distinguish between the catalytic activity of the dye molecule because of its position in the surface of the semiconducting lattice and the catalytic activity of the dye molecule because of its own chemical structure. Experiments in which single dye molecules act as catalysts or sensitizers, for example, on studies of homogeneous catalytic reactions in the liquid phase, would do this.

Oxidation of isopropyl alcohol is a convenient reaction, because the alcohol is the solvent for the dyes and reactions of other solvent molecules can therefore be avoided. Earlier investigations¹⁻⁴ have led us to choose triphenylmethane dyes as catalysts.

The reaction is carried out in small 'Pyrex' flasks containing 12 ml. of the solution of the dyes in isopropanol and 20 ml. oxygen at atmospheric pressure. The concentration of the dyes is 10⁻⁶ moles/ml. The flasks are mounted in a water thermostat and are illuminated by 40 W light bulbs. At intervals of several hours 1 µl. of the reacting solution is removed by a Hamilton microlitre syringe and is analysed in a 116 E Perkin-Elmer gas chromatograph (carrier gas He, 99.995 per cent from Linde; carbowax column) with a Sargent recorder (2.5 mV).

It was found that no reaction of the isopropanol occurs either in the dark or in the light unless a dye is added, but no dark reaction can be seen with any dye. No reaction decomposition or oxidation products of the dyes can be seen in the chromatogram; however, a slight decomposition of some of the dyes is seen from ultraviolet spectra of the solution. The reaction occurs according to the equation



CO₂ is also formed to a very limited extent (about 1/100 of the amount of acetone), but it is not clear whether this is from a parallel or from a subsequent reaction. Acetone

undergoes no reaction when used as solvent for the dyes.

The photocatalytic activity of the dyes at 50 and 70° C is shown in Table 1 in terms of maximum amounts of acetone formed after 48 h. A maximum of 50 units corresponds to 2 per cent conversion of isopropyl alcohol. In addition, the semiconductor properties of the solid dyes^{5,6} and the fluorescence of the molecules⁷ are given in the table. The first four dyes belong to the fluorane type (triphenylmethanes with an oxygen bridge). With the exception of erythrosine, they exhibit a high activity at 70° C and less activity at 50° C. With one exception, the catalytic active dye molecules form *p*-conducting crystals; all molecules are fluorescent.

The second group (5, 6) are molecules of the phenazine type, a nitrogen atom forming the bridge between the phenyl rings. They are clearly fluorescent vacuum semiconductors, with no catalytic activity. The molecules of the third group (7-11) are the triphenylmethane dyes without bridge atom. Again, they have no catalytic activity, are *n*-semiconductors and do not fluoresce. The phthaleins of the fourth group (12-14) have features similar to those of the triphenylmethanes.

The last three dyes are not triphenylmethane dyes and these results will not be discussed here.

At the present state of investigation, we may conclude that the catalytic activity of the dye molecule is connected with the mobility of the charge carriers in the ground state and with the immobility of the charge carriers in the excited state⁴, these being the properties causing *p*-conductivity in a molecular lattice. The interaction between substrate molecule and sensitizer molecule may be related to the charge transfer from molecule to molecule in the semiconductor lattice. As the activity of the fluorescent molecules shows, catalytic activity is also connected with the existence of a long living triplet state of the molecule¹⁻³.

As shown by the example of the phenazine dyes, however, the existence of a fluorescing triplet state is not a sufficient criterion for catalytic activity. We cannot yet say whether the *p*-conductivity or the oxygen bridge is the essential parameter, but both may be concerned. Furthermore, chemical aspects have to be considered: the iodine substituents of erythrosine are rather large and the low activity of erythrosine may be caused by sterical hindrance.

More complete conclusions cannot yet be drawn and we are now studying the various problems in more detail.

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Table 1

Dye (10 ⁻⁶ moles/ml.)	Acetone yield after 48 h		Conductivity <i>p</i> , <i>n</i> ⁵	O ₂ , V ⁶	Fluor- escence ⁷
	50° C	70° C			
1 Fluorescein	5	53		O ₂	+
2 Eosine	3	43	<i>p</i>	O ₂	+
3 Erythrosine	2	2	<i>p</i>	O ₂	+
4 Rhodamine B	2	52	<i>p, n</i>	V	+
5 Phenosafranine	—	—	<i>p</i>	V	+
6 Safranine T	—	—		V	+
7 Fuchsin	—	—		V	—
8 Malachite green	—	—	<i>n</i>	V	—
9 Methyl violet	—	—		V	—
10 Crystal violet	—	—	<i>n</i>	V	—
11 Aniline blue	1	—		V	—
12 Phenolphthalein	—	—			—
13 Phenol red	—	—			—
14 Bromphenol blue	—	—			—
15 Bromanil	2	2			—
16 Ethyl red	—	—			—
17 Pinachrome blue	1	—			—

Kerr Effect in Polymers

RECENT observations on bulk samples of polystyrene and polymethyl methacrylate have shown the existence of a Kerr-type electro-optic effect in these materials, the application of an electric field to the sample giving rise to a change in optical refractive index which is proportional to the square of the applied field, for light polarized normally to the applied field.

The observations have been made by constructing a Fabry-Perot interferometer cell with the polymer sample as the optical medium^{1,2}. The interferometer mirrors serve in addition to their normal function as electrodes for the application of a d.c. field of approximately 10^6 V/m, with a superimposed low-frequency a.c. field of similar magnitude. The effect of the two fields is to change the optical path length between the mirrors, and hence to amplitude-modulate a helium-neon laser beam passing through the electro-optic cell. Detection of the signal is by a photo multiplier tube and suitable electronic amplifying and display equipment.

The estimated magnitude of the Kerr coefficient for PMMA at 1 kHz is about 10^{-12} mV⁻² (10^{-5} cm/statV²), which is about half the value quoted in the literature for nitrobenzene. The corresponding figure for polystyrene appears to be considerably larger, but has not yet been measured. A curious feature of the effect is that in both materials it is associated with a relaxation time of about 10^{-4} s; in the case of PMMA there is a strong resonance at 15 kHz, followed by a rapid fall to near zero, while for polystyrene the modulation depth decreases steadily as the frequency is increased beyond about 5 kHz, without showing a resonance peak.

The possibility that this behaviour could be due to diaphragm resonances of the samples has been eliminated by repeating the experiments on disks of different thicknesses and diameters.

So far the experiments have only been carried out on uncharacterized materials at room temperature. The relaxation frequency we have observed does not seem to correspond to any other known room temperature relaxations in the dielectric properties of these materials, but a recent survey by Block and North³ reports similar relaxation frequencies for dielectric properties of a rod-like polymer (in this case poly- γ -benzyl-L-glutamate) in solution, the frequency being proportional to the reciprocal of the square of the molecular weight of the polymer. We hope to report later on the influence of molecular weight on our observations, when suitable characterized samples are available.

Some theoretical studies and a considerable amount of experimental work on the Kerr effect in various polymers in solution have been reported⁴⁻¹⁵, but our observations seem to be the first to be reported on solid polymer samples.

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Structure of Anomalous Water and Its Mechanism

SINCE the discovery of "anomalous water"¹, several models²⁻¹² have been proposed for its structure. I derive here a model based on present experimental knowledge, and then explain its mechanism.

With sp^3 orbitals for oxygen, the structure of water includes two "long pairs", which may be the media for hydrogen bondings. In Fig. 1a, we assume that such water molecules may be polarized by two small (structure making) cations, stretching one of the O—H bonds. The stretched hydrogen may become more positively charged and act as a cation to polarize the neighbouring water molecule and produce a chain effect. This description fits the discovery of a chain of water molecules in the field ionization of water¹³. The above mechanism may be regarded as analogous to the idea of "localized hydrolysis" of Robinson and Harned¹⁴ in aqueous electrolyte solutions. If the charge of the cation were strong, the stretched hydrogen might be ionized and leave the hydroxyl group attached to the cation with species like $[(HO)_nCu(OH)_2Cu(OH_2)_n]^{2+}$ (ref. 15), and $[(H_2O)_4Al(H-OHO-H)_2Al(OH_2)_4]^{4+}$ (ref. 16). This approach is consistent with the fact that the Raman spectra of water in concentrated ionic solutions of a wide range of salts¹⁷ as well as anomalous water³ show nearly identical peak frequency shifts from the $3,400\text{ cm}^{-1}$ band of normal water.

In our model for anomalous water, the silicon ions at the surface inside the quartz capillary induce with charge

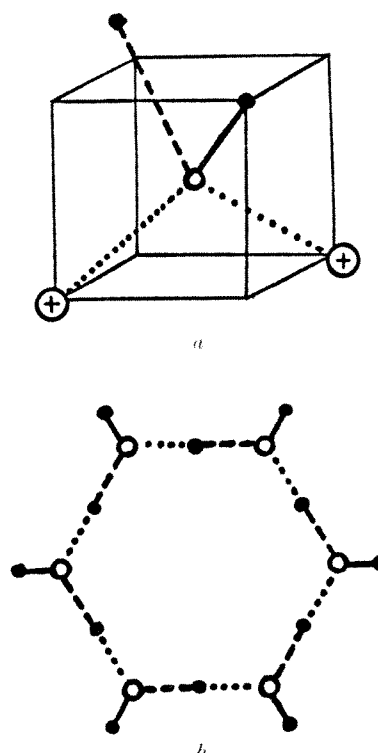


Fig. 1. a, The structure of a water molecule, with sp^3 orbitals for oxygen, being polarized by two positive charges and stretching one of its O—H bonds. \circ , Oxygen; \bullet , hydrogen; \oplus , small cation; —, unstretched O—H bond; ---, stretched O—H bond; \cdots , lone pair of oxygen. b, The arrangement of a six-membered ring formed by charge induced effect.

the arriving water molecules into forming a water molecular chain. For the small size of the capillary (2 to 4 μm in diameter), however, the chance of forming a ring is greater. According to Hertl and Hair's study of water adsorption on silica⁷, a six-membered ring is indicated. Molecular orbital calculations from first principles have also predicted that cyclic structures are the most stable¹⁸. Allen and Kollman⁹ have derived the six-membered ring arrangement theoretically. In the present model, the arrangement of the ring is shown in Fig. 1b, which can be seen as an equilibrium arrangement maintained by six hydrogens and six hydroxyl groups. By maintaining the sp^3 orbitals for oxygen, the ring is formed in the shape of a "chair" as shown in Fig. 2a. The ring may move on the surface.

In Fig. 2a, the dash-lines enclosing the ring are in the shape of a half-height pyramid with the (111) plane as its base. The sides of this base and its height are $(4\sqrt{6}/3)R$ and $(4/3)R$ respectively. R is the O-to-O distance of the

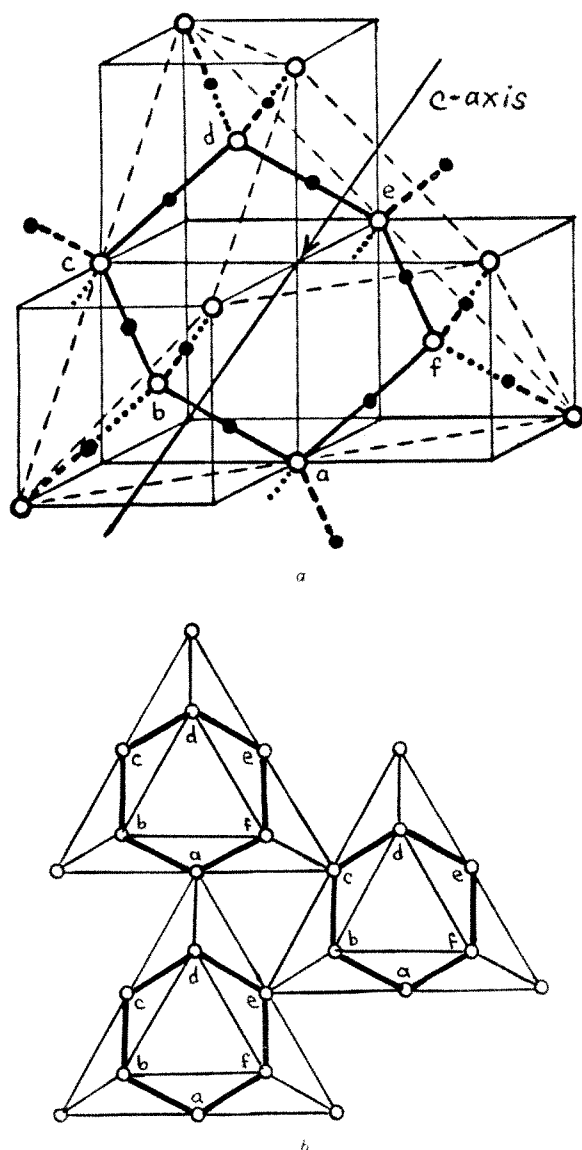


Fig. 2. *a*, The arrangement of a six-membered ring according to the sp^3 orbitals of oxygens. Only the oxygens are shown. The oxygens are labelled and the ring is indicated by the thick solid lines. The unstretched O—H bond is represented by the thick dashes. The surrounding thin dash-lines in the shape of a half-height pyramid with the (111) plane as its base are a ring unit. *b*, The top view of three ring units hydrogen bonded together to form a layer.

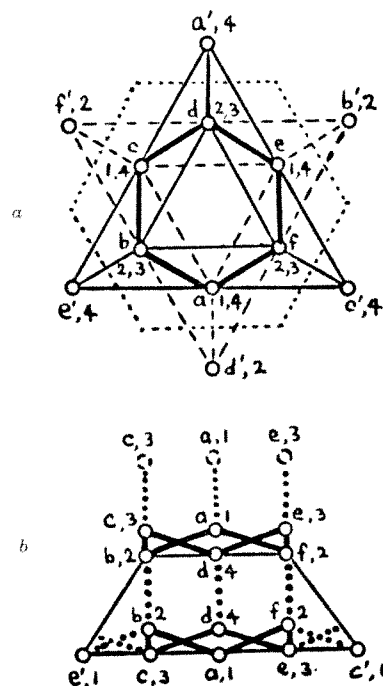


Fig. 3. The stacking of the six-membered rings into layers. Only the oxygens are shown. The sequences of layers for the labelled oxygens are indicated. *a*, Top view; *b*, side view. Its unit cell is a hexagon.

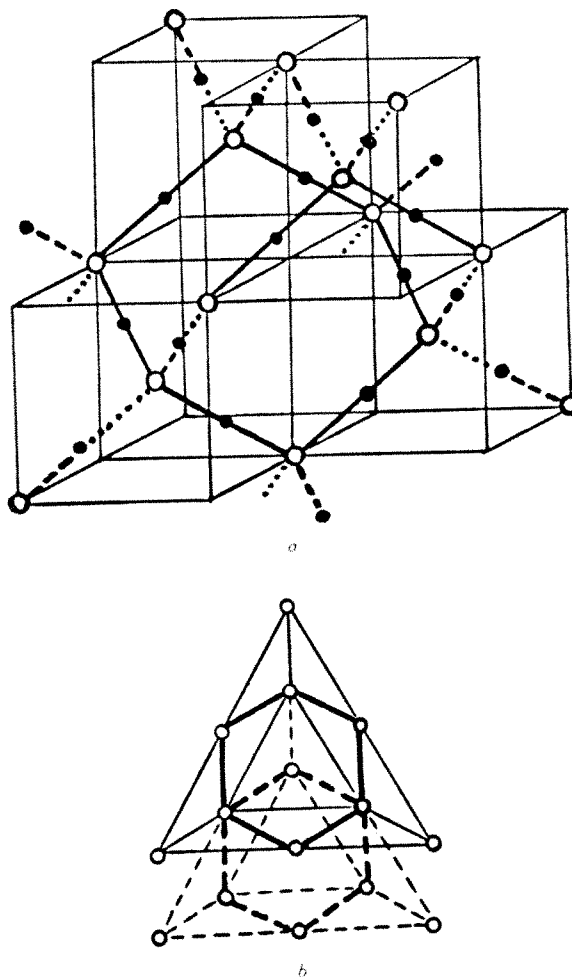


Fig. 4. One way of stacking the rings into (C9) type structure. *a*, The unit cell; *b*, the scheme of layer stacking.

ring. Fig. 2b shows the top view of this half-height pyramid and demonstrates how the rings can join together into a layer. If the ring in Fig. 2a rotates $2\pi/6$ about its *c*-axis and sits on top of another ring at its original position, the layers can be stacked together as shown in Fig. 3. It is assumed that, when the ring is isolated, the hydroxyl groups are just like any non-hydrogen bonded hydroxyl group. When bonded into layers, the hydrogen of these hydroxyl groups is shared by two oxygens and each oxygen is surrounded by four hydrogens. Equilibrium may be reached by forming the in-between ring hydrogen bonds with the same symmetry as those within the rings. Such a stacking, as in Fig. 3, has a hexagonal unit cell of $a = (2\sqrt{6}/3)R$ and $c = (8/3)R$ for twelve molecules. All the other ways of combining the rings give a structure of the *anti* β -Cristobalite SiO_2 (C9) type (diamond (A4) type structure if we consider only the oxygens), with $a = (4\sqrt{3}/3)R$ for eight molecules. Figs. 4 and 5 show two of them. Whichever way the rings are stacked, the average volume for a ring of six molecules is always the same, being $(16\sqrt{3}/3)R^3$. Although it has been suggested⁴ that the value of *R*, the O-to-O distance, is 2.3 Å, similar to that of F...H...F, this value has since been found to be wrong. Nakamoto and co-workers¹⁹ have established a relationship between the stretching frequencies of H and the X...H...X distances in hydrogen bonds which shows that there are two separate curves for the O—H...O and F—H...F hydrogen bonds. From the reported stretching frequency of 1,595 cm^{-1} for anomalous water⁴, one may estimate its O-to-O distance as 2.4 Å from the plots of Nakamoto and co-workers¹⁹. They have also found that, in strong hydrogen bonds, the O—H distance increases exponentially as the O—H...O distance decreases, suggesting that for hydrogen bonds of ~ 2.45 Å the hydrogen will be centred. By taking the O-to-O distance (*R*) as 2.4 Å, the present model gives the density of the anomalous water as 1.4 g cm^{-3} , which is equivalent to the largest experimental figure²⁰. From the same known stretching frequency⁴ of 1,595 cm^{-1} , one may also estimate the "hydrogen bond" energy (per bond unit) as -31 kcalories mol^{-1} (-130 kJ mol^{-1}) from the relationship established by Lamberts and co-workers²¹⁻²³.

The anomalous water is therefore formed as six-membered rings in a "chair" shape, hydrogen bonded to each other into a close-packed diamond-like arrangement. The O-to-O distance is taken as 2.4 Å and the hydrogen is centred between the two oxygens. Each oxygen is in turn surrounded by four hydrogens. The hydrogen bonds in anomalous water are believed to be charge induced and strong. The whole network may be regarded as an equilibrium arrangement maintained by H^+ and O^{2-} with some OH^- at the boundary of the column—these OH^- groups may form hydrogen bonds with the oxygens available at the silica surface, which then eliminate the non-hydrogen bonded OH^- groups. Such a structure for anomalous water probably represents the most ordered structure of water. Its ordering mechanism may be in evidence near the water-solid interfaces discussed by Drost-Hansen²⁴ and the super-cooled water found by Frank^{25,26}. One may certainly find the same mechanism in clay, soil, tissue and cells. It may also solve the question of why water can reach the top of a very tall tree.

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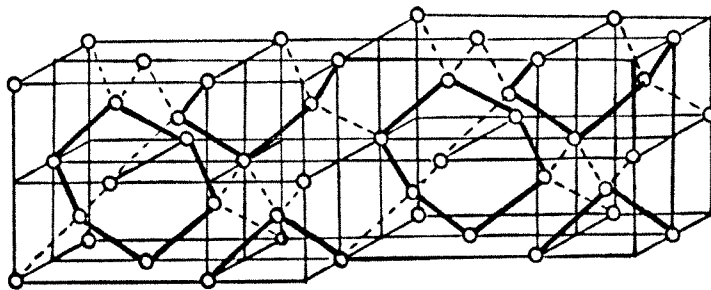


Fig. 5 Another way of hydrogen bonding the rings into (C9) type structure. The basic unit cell is the same as in Fig. 4a.

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Pyrite Crystals in the Parenchyma Cells in Wood of Fossil Root

VERY small octahedral crystals (1 micron–20 micron) of iron pyrite have been found in the ray and wood parenchyma cells in parts of a fossil root of Oligocene age. The black humified wood is elsewhere partially replaced by veins of pyrite, as commonly seen in fossil wood of this



Fig. 1. Radial section of wood showing horizontal tiers of ray cells containing crystals. A vertical row of wood parenchyma cells is arrowed at top right ($\times 60$).

sort. The wood, illustrated in Figs. 1 and 2, was taken from one of many roots observed in the Hordle Cliff section of the Lower Headon beds near Milford-on-Sea, Hampshire. Similar roots occur in exposures of these beds at Totland Bay, Isle of Wight, and in both places the roots are in the position of growth¹. All the wood that we have examined anatomically is of a single type and, although some of the finer diagnostic features are not well preserved, it is closely similar in structure to the wood of *Taxodium distichum* (swamp cypress). We cannot, however, ignore the possibility that the roots belonged to the Tertiary *Sequoia couttsiae* which is prominent among the plants identified from other remains in the Lower Headon beds². A full description and discussion of the identity of our material will be presented elsewhere.

Microscopic pyrite, in the form of crystals and framboidal spherules, is common in dark shales and muds, and where it occurs in recent sediments it is a clear indication of early syngenetic formation of iron sulphide. A feature of especial interest is the constant association of the pyrite in these sediments with organic material, and pyrite is often found within the walls of diatoms, foraminifera, and plant spores and pollen grains^{3,4}. Coleman has recently described⁵ the replacement by pyrite of hollow rotted rootlets in freshwater clays of the Mississippi delta.

The precise role of organic matter in the early formation of pyrite is difficult to assess. There can be little doubt that the cells of animals and plants do not normally contain sufficient sulphur to account for the quantities of sulphides produced in sediments; and decomposing organic matter would not be capable of reducing sulphate to any appreciable extent in the absence of sulphur bacteria⁶. It has been suggested that pyrite spherules are the remains of sulphur bacteria or some other organism which deposited sulphides in their cells⁷, but pseudomorphic bacterial cells have not been seen in the pyrite of spherules, nor was any organism found to be directly precipitating pyrite in Recent sediments which contained organic material in which pyrite had formed⁸.

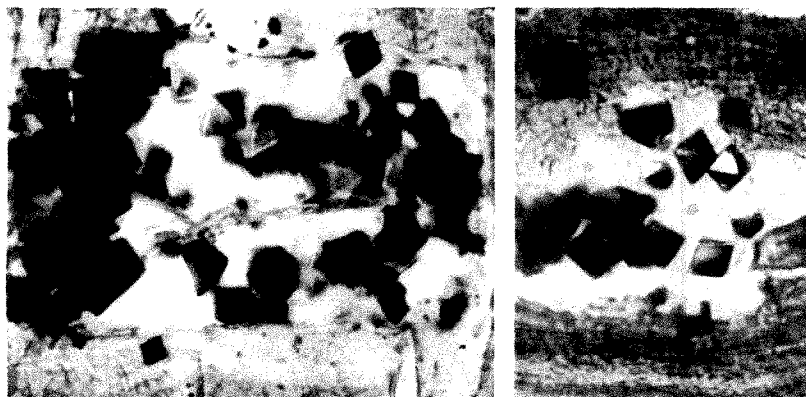


Fig. 2. Crystals at higher magnification ($\times 600$).

Preferential mineralization of organic material is clearly significant in fossilization⁹, and has some relevance to the genesis of certain ore deposits⁹. Emery has stressed¹⁰ the importance of the creation of a microenvironment within shells of decomposing diatoms, foraminifera and the like, which become infilled with pyrite. The localization of masses of tiny pyrite crystals in the parenchyma cells of our fossil root certainly supports the view that the microenvironment within the dead or dying tissue is an important factor in the early formation of pyrite. The parenchyma cells are the only cells which contained protoplasm, and these cells have provided the essential conditions that promoted the crystallization or precipitation of iron sulphide from the solution infiltrating the rotting root. The actual presence of sulphur bacteria

within these cells would not be necessary for this to have taken place.

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BIOLOGICAL SCIENCES

Satellite DNAs in Crustacea: Two Different Components with the Same Density in Neutral CsCl Gradients

SINCE the first reports of satellite DNAs in the Jonah crab, *Cancer borealis*¹, and in the mouse², satellite DNAs have been described for a number of animal species, both vertebrate and invertebrate. We now report our findings on the satellite DNAs of five species of Crustacea. We find at least one and in some instances two DNA satellites in each species and were surprised to discover that two distinctly different satellite DNAs band at the same density, 1.677 g/cm³, in CsCl. DNAs banding at this density are usually assumed to be "crab poly d(A-T)", the satellite DNA composed of more than 90 per cent alternating adenylate and thymidylate residues^{3,4}. It now seems that this cannot be assumed without further characterization of the DNA species having that density. We emphasize that the satellites described in this note are not mitochondrial in origin: even though we find a mitochondrial DNA of $\rho = 1.688$ g/cm³ in each of the five species, the quantity is too small to be detectable in a "total" DNA

preparation. These data will be presented elsewhere.

The animals used were the Bermuda land crab, *Gecarcinus lateralis*; the edible blue crab, *Callinectes sapidus*; the spider crab, *Libinia dubia*; the Jonah crab, *Cancer borealis*; and the lobster, *Homarus americanus*. DNA was isolated by the method of Marmur⁵ with modifications described previously⁴. We used chiefly gonads or haemocytes. The purified DNAs were analysed by their buoyant density in CsCl as determined with a model E analytical ultracentrifuge or by their melting behaviour registered by a Gilford recording spectrophotometer. Although the total amounts of isolatable DNA varied from one tissue to another, as may be expected, the relative quantities of satellite DNAs compared with main band DNA were fairly constant and characteristic of the animal species.

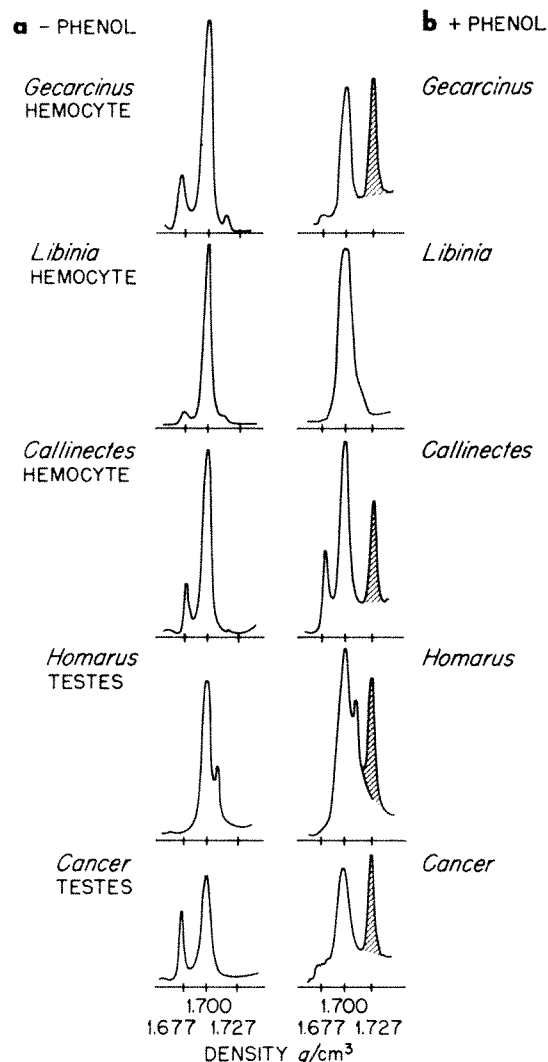


Fig. 1. Microdensitometer tracings of CsCl isopycnic density gradients of DNA before and after treatment with phenol. Samples were centrifuged in the model E analytical ultracentrifuge at 44,770 r.p.m. for 20 h at 25° C. *a*, Minus phenol treatment: DNA was purified as described from the species of animal indicated. *b*, Plus phenol treatment: samples of the purified DNAs depicted in *a* were brought to 0.1 M NaCl, 0.05 M EDTA (pH 8) in the cold. SDS and 5 M NaCl were added to 2 per cent and 1 M, respectively. After the addition of an equal volume of redistilled phenol (71–74° C fraction), samples were shaken for 1 h in the cold and centrifuged; the aqueous phases were then removed and dialysed in the cold against SSC/10 to remove phenol. *M. luteus* DNA was used as a marker, $\rho = 1.727$ g/cm³.

Buoyant density patterns for the five species are shown in Fig. 1*a*; the data are also summarized in Table 1, which includes the calculation of the percentage of each satellite present. The buoyant density determinations for each species were calculated with reference to either a light or heavy marker. For a light marker, we used "crab poly d(A–T)", $\rho = 1.677$ g/cm³, isolated from the DNA of *Gecarcinus* by two sequential centrifugations in preparative CsCl isopycnic gradients^{4,6}. For a heavy marker we used *M. luteus* DNA, $\rho = 1.727$ g/cm³. The main DNA band had a density of 1.698–1.701 g/cm³ in each species. The DNA from each species was also centrifuged without markers so that satellites could be detected and their concentration relative to the main band DNA calculated (see Fig. 1*a*).

Satellites rich in G+C are found in *Gecarcinus* as reported previously⁴, in *Libinia*, and possibly in *Callinectes*; one is especially prominent in *Homarus*. The buoyant densities of these dense satellites differ from one

species to another (Table 1). A method for their preparative isolation has been described by D. E. G.⁷.

A light satellite ($\rho = 1.677$ g/cm³) is apparent not only in *Gecarcinus* and *Cancer* sp.^{1,4,8}, but also in *Libinia* and *Callinectes*. Whereas others^{9,10} have shown and we have confirmed that glycogen also bands in this CsCl gradient region (indeed, such glycogen bands may contain as much as 98 per cent of the label from ¹⁴C-thymine incorporated into tissues¹¹), the light satellites from all four of the crabs are sensitive to deoxyribonuclease and resistant to amylase, and their absorbances increase on heating. Although these satellites have the density characteristic of the "crab poly d(A–T)" and three of the four have other characteristics typical of this material, the light satellite of *Callinectes* seems to be a special case. This first became evident when purified DNA preparations were exposed to phenol^{12–14}, which extracts "crab poly d(A–T)"¹⁵ and synthetic poly d(A–T)¹⁶. The light satellites from all species except *Callinectes* are lost on treatment with phenol (Fig. 1*b*).

Table 1. CRUSTACEAN DNAs: THEIR BUOYANT DENSITIES IN CsCl AND PERCENTAGE OF TOTAL DNA

	Buoyant density (g/cm ³), with per cent total DNA in parentheses		
	Light	Main band	Heavy
<i>Gecarcinus lateralis</i>	1.677 (18)	1.701 (79)	1.721 (3)
<i>Libinia dubia</i>	1.675 (6)	1.698 (92)	1.714 (2)
<i>Callinectes sapidus</i>	1.676 (15)	1.698 (85)	
<i>Cancer borealis</i>	1.676 (23)	1.698 (77)	
<i>Homarus americanus</i>		1.700 (90)	1.713 (10)

Densities in neutral CsCl were calculated from microdensitometer tracings of analytical ultracentrifugations in a model E centrifuge. "Crab poly d(A–T)" or *M. luteus* DNA was used as a marker. Except for *Libinia* each calculation is the average of two or more model E analyses of two or more DNA preparations. Percentages were determined from areas of Joyce-Loebl microdensitometer scans with a planimeter or by tracing the peaks and weighing cut out areas.

Although "crab poly d(A–T)" bands at a density of 1.677 g/cm³ in CsCl, it has long been known that the linear relationship between G+C content and buoyant density does not hold for that satellite¹⁷. Such a buoyant density is actually consistent with DNA having a G+C content as high as 27 per cent¹⁸. We have obtained further information on G+C content by determining the thermal dissociation properties of the various DNAs. The results with *Libinia*, *Gecarcinus* and *Cancer* DNAs were as expected from previous data on the dissociation of "crab poly d(A–T)" at elevated temperatures⁴; the light satellite DNA in each case melted at 52° C in 0.015 M NaCl–0.0015 M sodium citrate (SSC/10). The results obtained with *Callinectes* and *Gecarcinus* are plotted in Fig. 2*a*. It is clear that the melting behaviour of the *Callinectes* satellite differs from that of the *Gecarcinus* satellite, for an 11° C difference in T_m can be seen in a single sample when the two DNAs are heated together (Fig. 2*a*). The small shoulder at 63° C on the melting curve of *Callinectes* DNA (Fig. 2*a*, inset) was shown by independent experiments to correspond to the d(A+T)-rich satellite (Fig. 2*b*).

In conclusion, satellite DNAs, which are both heavier and lighter than main band DNA, have been found in five crustacean species. A light satellite resembling "crab poly d(A–T)" in CsCl gradients has been found in the blue crab, *Callinectes*, but by various criteria this satellite has been shown in fact to be different from "crab poly d(A–T)". The measured T_m (Fig. 2*a*) of the (A+T)-rich satellite from *Callinectes* is that expected for a DNA with a G+C content of 27 per cent, and such a DNA would also have a density in neutral CsCl of 1.677 g/cm³ (ref. 18). Clearly, satellite DNAs of $\rho = 1.677$ g/cm³ (ref. 10) require additional analyses to determine their base compositions.

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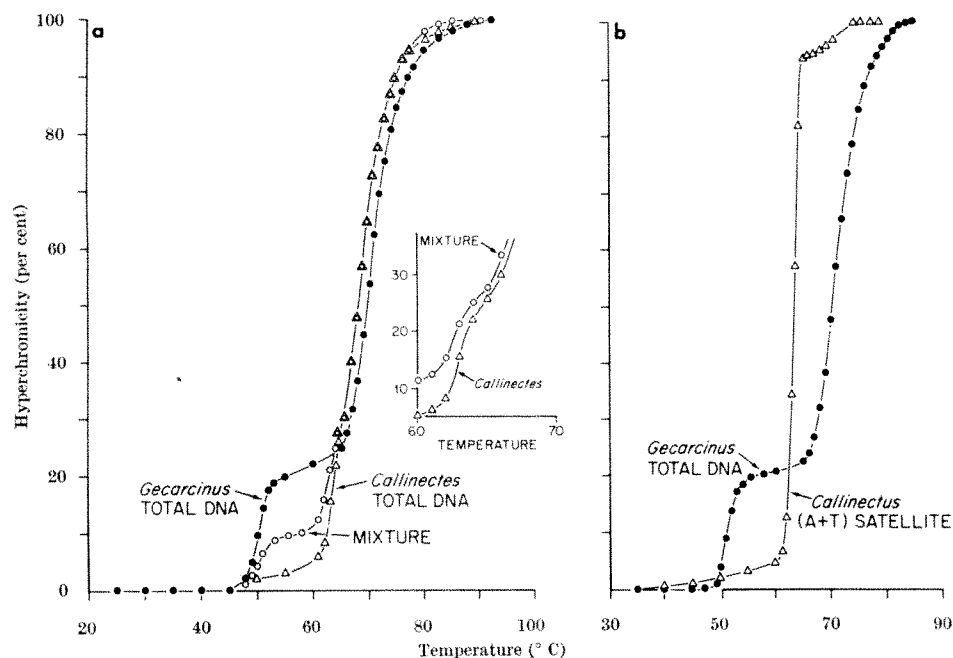


Fig. 2. Thermal dissociation of DNA of *Gecarcinus* and *Callinectes*. a, 40 μ g DNA isolated from the haemocytes of *Callinectes* (Δ — Δ); 20 μ g of DNA isolated from the testes of *Gecarcinus* (\bullet — \bullet); and a mixture of the two DNAs, 20 μ g *Callinectes* and 10 μ g *Gecarcinus* (\circ — \circ), were heated in SSC/10 in stoppered 1 ml. cuvettes in a Gilford spectrophotometer programmed for a 2° C temperature increase per min. The inset in a is an enlargement of the temperature range from 60–70° C. b, *Callinectes* (A+T)-rich satellite DNA was greatly enriched by centrifugation of total DNA in a preparative gradient of 7.7 M CsCl (refs. 4 and 6). 49 μ g of the material in the light band was concentrated to a volume of 1 μ l, dialysed against SSC/10, and its thermal dissociation was monitored (Δ — Δ). The hyperchromicity at 71° C arises from the main band DNA contaminating the preparation. Total DNA of *Gecarcinus* was melted as a control (\bullet — \bullet).

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Dissociation of Haemoglobin Chesapeake into Subunits

AMONG the large number of human haemoglobin variants of known structure, most are functionally normal. Haemoglobin Chesapeake (α_2 92 Arg→Leu β_2) is the first one found in association with elevated whole blood oxygen affinity and familial erythrocytosis. By contrast with haemoglobin A, isolated haemoglobin Chesapeake displays a six-fold increase in oxygen affinity and a marked reduction in haem-haem interaction (Hill's coefficient $n = 1.3$), but a normal Bohr effect². In the past two years, other variants with similar properties have been reported, but none has been studied as extensively as haemoglobin Chesapeake. These mutants provide considerable information relating the structure and function of haemoglobin, for their amino-acid substitutions are likely to be at sites crucial to the normal function of the molecule. It is of considerable interest that in four of these haemoglobins (Chesapeake, J-Capetown, Yakima and Kempsey), the substitutions are located in the area of contact between the α_1 and β_2 chains³. Three-dimensional models constructed from the X-ray crystallographic data of Perutz and associates indicate that, during oxygenation, there is considerable movement of the β chains relative to the α chains at this interface⁴. It is likely that the symmetrical dissociation of oxyhaemoglobin into $\alpha\beta$ dimers also occurs at the $\alpha_1\beta_2$ contact areas^{5,6}.

The extent of subunit dissociation of haemoglobin Chesapeake, compared with that of haemoglobin A, was studied by analytical ultracentrifugation. Previously, gel filtration data indicated that the dissociation of the liganded forms of haemoglobin Chesapeake into subunits was qualitatively less than haemoglobin A (ref. 7). Haemoglobins Chesapeake and A were prepared from a fresh haemolysate on carboxymethylcellulose as described by Charache *et al.*¹. Our results enable us to compare A and Chesapeake haemoglobins isolated on the same column. A Spinco model E analytical ultracentrifuge was

used, with a Corning 2-61 red filter and Kodak 1-D red-sensitive plates. Sedimentation velocity measurements were done at 50,740 r.p.m., 15° C, on 0.2 per cent phosphate-free haemoglobin solutions in 30 mm aluminium cells (Table 1). The use of a negative wedge window allowed specimens of haemoglobins A and Chesapeake to be run simultaneously. Subunit dissociation was enhanced in the presence of increasing concentrations of NaCl. Both the oxy and carboxy forms of haemoglobin Chesapeake had relatively higher $S_{20,w}$ values than haemoglobin A (Table 1), indicating that in high NaCl concentrations, the liganded forms of haemoglobin Chesapeake dissociated less readily into subunits. By contrast, the $s_{20,w}$ values of the deoxy form of the two haemoglobins were the same in 2.0 M NaCl, although higher than the carboxy values. Because of its high oxygen affinity, haemoglobin Chesapeake could not be maintained in the deoxygenated state during the centrifuge run unless 1.0 mM sodium dithionite was present. To minimize possible side reactions⁸, the addition of dithionite to deoxyhaemoglobin and the transfer to the cell were performed in strict anaerobic conditions. The $s_{20,w}$ values of deoxyhaemoglobin A in 2.0 M NaCl were the same whether or not dithionite was present. The data (Table 1) confirm earlier observations that deoxyhaemoglobin A dissociates less readily than the liganded haemoglobin^{9,10}.

Recently, Edelstein and Gibson have shown that because of incomplete permeation of salt inside the haemoglobin molecule, a flotation effect reduces sedimentation in high NaCl concentrations¹¹. Thus these $s_{20,w}$ values in 1.0 and 2.0 M NaCl overestimate the extent of subunit dissociation. Nevertheless they show qualitative differences between the liganded forms of haemoglobins A and Chesapeake.

To examine the subunit dissociation of these haemoglobins at a more physiological ionic strength (0.1 M NaCl), a much lower protein concentration was required. Use of interference optics allowed a haemoglobin concentration of 0.03 per cent. Weight average molecular weights were determined in triplicate by the method of Yphantis, using a 12 mm double sector aluminium epoxy cell¹². Runs were done at 25,980 r.p.m. for 16 h at 10° C. The spectra of carboxyhaemoglobin in the cell following centrifugation revealed no auto-oxidation. The mean molecular weight of haemoglobin Chesapeake was 64,900, significantly higher than the mean value of 60,800 obtained for carboxyhaemoglobin A ($0.01 < P < 0.05$).

Intact α chains of carboxyhaemoglobins A and Chesapeake were prepared by the method of Bucci and Fronticelli¹³. Mercury was removed from globin sulphhydryl groups by thioglycollate¹³. Spectrophotometric oxygen equilibria experiments were done on 0.4 per cent haemoglobin solutions in 0.1 M NaCl-0.05 M bis Tris buffer (pH 7.2) at 10° and 20° C. The mutant α chains of haemoglobin Chesapeake consistently showed a somewhat lower oxygen affinity than normal α chains from haemoglobin A. This difference was not related to any detectable aggregation of α chains as shown by the $s_{20,w}$ values in Table 1. Thus the high ligand affinity of the Chesapeake tetramer does not seem to be related to the immediate haem environment of the mutant α chain, but rather to interaction between the subunits.

These results indicate that liganded forms of haemoglobin Chesapeake dissociate into subunits less readily

than haemoglobin A. Because the amino-acid substitution in haemoglobin Chesapeake is at the $\alpha_1\beta_2$ contact area, these data strengthen the evidence that the symmetrical dissociation of the liganded tetramer into $\alpha\beta$ dimers is at this interface. It is likely that the increased energy of binding between subunits of the liganded form of haemoglobin Chesapeake favours this conformational isomer over the deoxy form and contributes to the high oxygen affinity of haemoglobin Chesapeake. The thermodynamic linkage between ligand affinity and subunit interaction has been stressed¹⁴⁻¹⁶.

According to recent data of J. Greer, difference Fourier of haemoglobins Chesapeake and A showed that the oxy forms differ extensively in conformation whereas the deoxy forms differ only in the known amino-acid substitution ($\alpha 92 \text{ Arg} \rightarrow \text{Leu}$)¹⁷. But the deoxy forms probably differ in conformation as well, as shown by sulphhydryl reactivity and ultraviolet difference spectra². Recent nuclear magnetic resonance data of Ho and associates indicate that both the oxy and deoxy forms of haemoglobin Chesapeake differ significantly from haemoglobin A (ref. 18).

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¹³C Nuclear Magnetic Resonance Spectrum of Gramicidin S-A, a Decapeptide Antibiotic

ALTHOUGH proton magnetic resonance spectroscopy has improved our knowledge of the structure and function of biopolymers, especially complex peptides and proteins¹, it has not yet solved such problems as determining the conformation of a protein in solution. The nearest approach has been the analysis of the spectrum of gramicidin S-A², a peptide of molecular weight 1,120 (Fig. 1). The technique used there is theoretically applicable to biopolymers of higher molecular weight, but the experimental difficulties are almost insurmountable.

We report here the ¹³C nuclear magnetic resonance (NMR) spectrum of gramicidin S-A and our assignment of the various ¹³C chemical shifts. Our data suggest that the study of ¹³C spectra is entirely feasible and, moreover,

Table 1. $s_{20,w}$ VALUES OF HAEMOGLOBINS A AND CHESAPEAKE 0.05 M BIS TRIS BUFFER pH 7.2

Form	NaCl (M)	A	$s_{20,w}$ Chesapeake
Carboxy	0.1	4.42	4.53
Carboxy	1.0	3.96	4.22
		4.02	4.30
Oxy	1.0	3.90	4.22
		3.76	4.00
Carboxy	2.0	3.14	3.37
		3.02	3.39
Deoxy	2.0	3.79	3.74
		3.90	3.92
Carboxy α chains	0.1	2.15	2.08

that ^{13}C spectra are more easily interpretable than proton spectra.

In naturally occurring molecules the predominant carbon isotope is ^{12}C which has no spin and does not exhibit magnetic resonance. Although the ^{13}C isotope has a natural abundance of only 1.1 per cent, it has a spin of $1/2$, and ^{13}C spectra of simple molecules³, steroids⁴ and some amino-acids^{5,6} have been recorded in enriched samples and at natural abundance. Nobody has yet reported the ^{13}C spectrum of a protein or any molecule comparable in size to gramicidin S-A, in natural abundance or otherwise.

We recorded the ^{13}C NMR spectrum of gramicidin S-A at 25.15 MHz with time averaging to enhance the signal to noise ratio. The spectrum extends over 160 p.p.m. of the applied field relative to ^{13}C S_2 as external standard. The complicating feature of ^{13}C -proton coupling was eliminated by noise decoupling and ^{13}C - ^{13}C interactions are not seen, for the chance that an individual ^{13}C atom will have another ^{13}C atom adjacent to it is approximately 1 in 10^4 . Each carbon thus appears as a singlet. There seems to be no evidence of ^{14}N - ^{13}C coupling, but this feature requires more study. Samples were run in 12 mm tubes with no spinning. Concentrations varied from 300–500 mg/2 ml.

The spectra are readily divided into well-separated regions: (a) carbonyl carbons (19–24 p.p.m.), (b) aromatic heteroaromatic carbons (50–70 p.p.m.), (c) C_α carbons (130–140 p.p.m.), (d) C_β carbons (150–165 p.p.m.), and (e) C_γ and methyl carbons (165–180 p.p.m.).

Fig. 2 shows the ^{13}C spectrum of gramicidin S-A in two solvents. Rigorous assignments of individual carbon atom resonances will require more exacting experiments, but all of the general features can be discerned. The ^{13}C spectrum of gramicidin S-A in either methanol or DMSO should have a total of twenty-eight lines, including nineteen from the side chain and C_α carbon atoms. In addition there should be four aromatic lines (ortho, meta, para and bridge phenylalanine atoms) and five carbonyl (C_0) lines. This can be regarded as agreeing with the results found if one accepts that (a) the ^{13}C DMSO peak obscures one line, (b) there are four lines in the 166–170 p.p.m. region in DMSO, (c) the four single lines at 170.0 p.p.m. (Fig. 2A), 167.5, 168.9 and 172.5 p.p.m. (Fig. 2C) are each caused by two carbon atoms whose lines are superimposed, and (d) one line in the C_0 region is doubled because of superimposition of two resonances. We thus obtain a complete carbon atom count for the whole molecule, assuming C_2 symmetry.

Our assignment of peaks in the spectrum to individual carbon atoms is based on a comparison of peak positions with the ^{13}C chemical shifts found for the N-acetyl amino-acid methyl esters. Individual peaks in the monomer spectra were assigned by comparison with the spectra of the amino-acids, both as observed^{5,6} and as calculated from Grant's rules^{7,8}. Agreement between observed and predicted chemical shifts for amino-acids is excellent, as shown in Table 1.

The ^{13}C spectra of the five amino-acids comprising gramicidin S-A (in H_2O) and their N-acetyl methyl ester derivatives are shown in Table 1. Although the peaks of the amino-acid and the ester do not superimpose, the differences observed are remarkably constant from amino-acid to amino-acid. All of the side chain resonances of the derivatives are found 1.5 ± 0.9 p.p.m. downfield from the corresponding resonances in the free amino-acids, presumably because of the difference in solvent bulk magnetic susceptibilities. The C_α carbon resonances, however, shift upfield 2.0 ± 0.5 p.p.m. because of charge redistribution in the derivatives.

Individual carbon assignments for the side chain and C_α carbon region are shown in Table 1. There was no difficulty in making general assignments, although in several cases minor ambiguities were apparent. None of these caused confusion. The lowest-field pair of lines

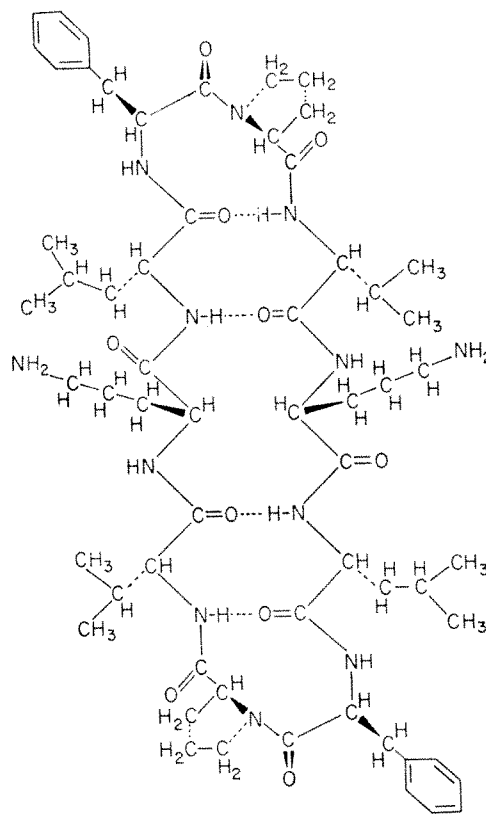


Fig. 1. Schematic drawing of the three-dimensional structure of gramicidin S-A, cyclo (-Phe-Pro-Val-Orn-Leu-), proposed from proton NMR studies². All ω angles are 0° except for Leu = 10° . The ϕ angles of Val, Orn, Leu = 30° , Phe = 150° . The ψ angles of Val, Orn, Leu = 0° , Phe and Pro = 130° .

clearly belongs to the proline and valine C_α carbon atoms but, because the monomer chemical shifts in this case are so close, it is not possible to decide with certainty which belongs to valine and which belongs to proline. Similar problems were encountered with the next pair of lines to higher field, and with the higher-field C_β and

Table 1. PEAK POSITIONS AND ASSIGNMENTS

Carbon	Amino-acid* predicted	Amino-acid†‡ observed	Derivatives§	Gramicidin S-A in DMSO	Gramicidin S-A in methanol
Pro α	125.5	132.6	134.0	132.6	130.3
Val α	127.3	132.6	134.7	135.7	131.9
Orn α	132.7	—	138.5	138.6	136.4
Phe α	—	136.9	138.6	141.4	139.6
Leu α	135.8	139.5	142.1	142.8	140.7
Pro δ	148.3	147.5	144.8	146.1	144.3
Orn δ	153.0	—	152.0	Solvent	150.4
Leu δ	153.2	153.0	151.0	154.1	151.5
Phe β	—	156.5	155.3	156.8	154.9
Orn β	164.7	—	162.0	161.3	160.2
Val β	167.4	163.8	162.1	162.8	161.2
Pro β	166.6	164.3	163.0	163.4	161.6
Pro γ	169.5	169.6	167.6	168.5	166.4
Leu γ	170.3	168.6	168.0	169.5	167.5
Orn γ	168.2	—	168.0	169.5	167.5
Leu CH_3	170.7	170.8	169.0	170.0	168.9
Leu CH_3	170.7	171.9	171.0	170.0	168.9
Val CH_3	176.5	174.9	173.0	173.6	172.5
Val CH_3	176.5	176.1	173.7	174.5	172.5

* From parameters in Grant and Paul⁷ and Horsley *et al.*⁸. Phenylalanine predictions are not possible from these parameters.

† From Horsley *et al.*⁸. Ornithine was not reported.

‡ All positions are chemical shifts in p.p.m. upfield from $^{13}\text{CS}_2$.

§ N-acetyl amino-acid methyl esters in DMSO.

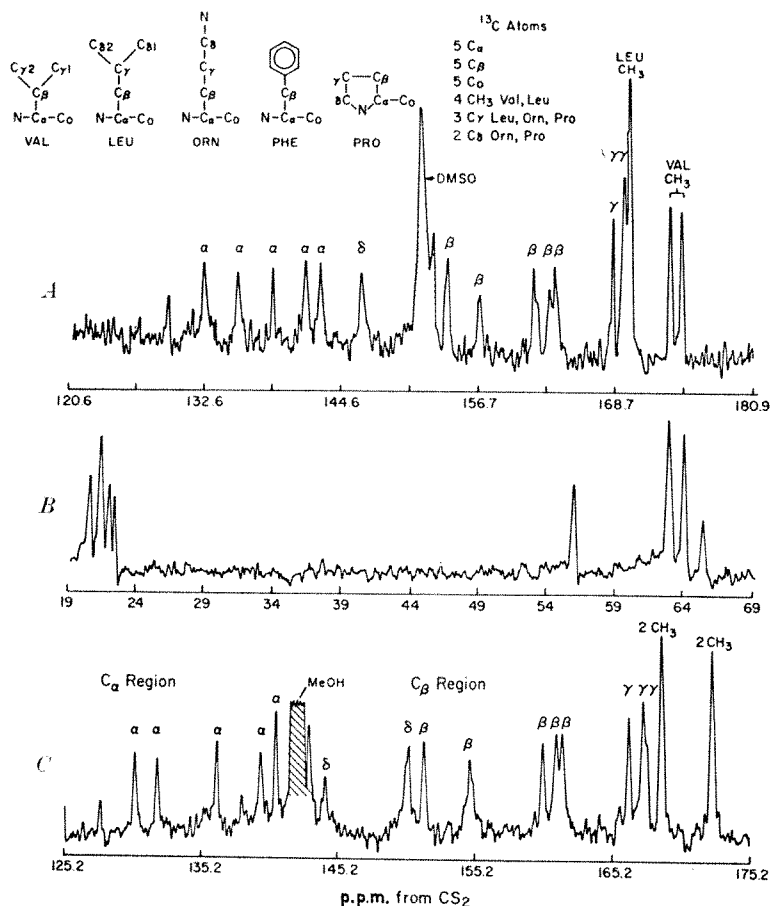


Fig. 2. The natural abundance ^{13}C NMR spectrum of gramicidin S-A. A, The high-field part of the spectrum in DMSO; B, the low-field part in DMSO; C, the high-field part in methanol. All chemical shifts are in p.p.m. upfield from $^{13}\text{CS}_2$ as external standard.

lowest-field C_β . Further, the three high-field C_β carbons are too close to one another to permit unambiguous assignments, and so are the three C_γ carbons. None of the chemical shifts is displaced significantly from the value found for the corresponding N-acetyl amino-acid methyl ester carbon. We now have enriched ^{13}C amino-acids available for selective incorporation studies to remove these difficulties.

Unfortunately, the carbonyl resonances fall within too narrow a range to be assigned at present. While carbonyl carbon resonances have been studied^{3,10,11}, insufficient is known for accurate predictions in this region. The phenyl-alanine lines may be assigned empirically as (low-field to high-field): bridge, ortho, meta, para.

The symmetry of gramicidin S-A is unequivocally C_2 from this spectrum, in agreement with the deduction from the proton spectrum². The five pairs of residues yield only five carbonyl, five C_α and five C_β chemical shifts. There is some interest in the two methyl group ^{13}C lines of valine. These groups are not magnetically equivalent, so two lines are to be expected, but the chemical shift difference between them is observed to vary from amino-acid to derivative to gramicidin. This can imply that the valine side chain has different average rotational conformations in the three cases. Proton spectra also revealed this difference². The $J_{\alpha\beta}$ proton coupling constant in valine alone is 6.5 Hz, but it is 9.0 Hz in gramicidin S-A, showing that in the latter the *trans* isomer predominates, that is, the isomer with the α and β protons *trans* to one another.

We now consider the relative heights and intensities of the various ^{13}C lines in the NMR spectra. There should be a general enhancement of all ^{13}C resonances on noise decoupling of protons because of the Nuclear Overhauser

Effect⁶; and the enhancement factor of 2.988 is theoretically possible wherever the ^{13}C relaxation mechanism is dominated by proton- ^{13}C dipole-dipole coupling⁹. Large deviations from this figure will be found only where the ^{13}C nucleus is not directly bonded to a proton or where the ^{13}C -H separation greatly exceeds that of a normal carbon-hydrogen bond. In our spectra of the N-acetylamino-acid methyl esters, the intensities of the ^{13}C lines are equal. This is not the case with gramicidin S-A. Because time averaging was necessary and variable RF power spectra were not taken, we are unable to explain these findings. We hope that the intensity relationships will help to identify chemical shifts⁹ and, when properly identified, yield information on relaxation times, transition probabilities and the molecular environment of the various carbon atoms in the molecule.

^{13}C NMR spectroscopy thus has strong advantages over proton spectroscopy in the study of complex peptides and, hopefully, proteins. In natural abundance, with noise decoupling of protons, the spectra obtained are much simpler than those for proton NMR. The simplicity of the spectrum and the near predictability of chemical shifts make assignments somewhat easier. The spectra are readily divided into well-separated regions: (a) carbonyl carbons, (b) aromatic-heteroaromatic carbons, (c) C_α carbons, (d) C_β carbons, and (e) C_γ and methyl carbons. Any slight overlap between these regions is not enough to interfere seriously with assignments. Specifically enriched ^{13}C spectra and spin-coupled and coherently decoupled spectra will confirm and complete the assignments.

^{13}C NMR also promises to lessen the problem of dipole-dipole line broadening of high molecular weight species such as proteins. Because of the smaller magnetic moment of the ^{13}C nucleus, ^{13}C line widths are theoretically only about one-sixteenth as susceptible to this form of line broadening as proton line widths.

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Release from Density Dependent Growth Inhibition by Proteolytic Enzymes

CONFLUENT chick embryo cells in culture grow much more slowly than do sparse cells in conditions where significant depletion of the medium is avoided¹⁻³. Because an important factor regulating growth seems to be the population density, this phenomenon is called density dependent inhibition of growth⁴. When a thin scratch is made in a slowly growing confluent culture of chick embryo cells, the cells adjoining the scratch begin to grow rapidly and

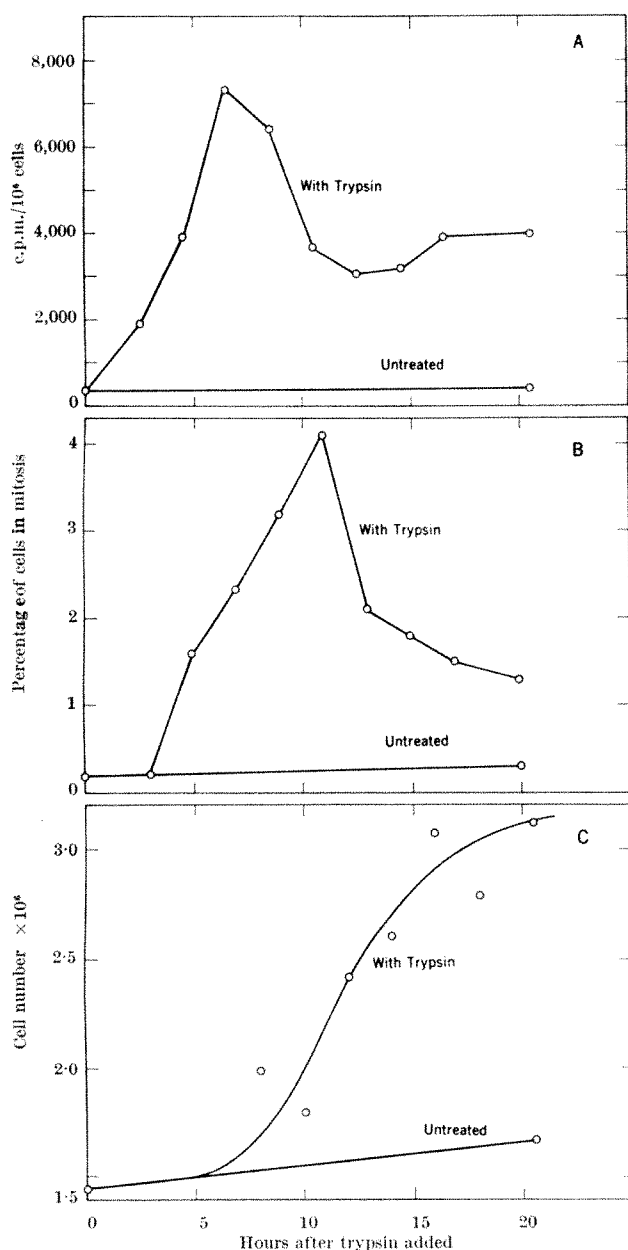


Fig. 1. Rate of incorporation of ³H-thymidine (A) and mitosis (B) and increase in cell number (C) in the first 24 h following the addition of trypsin. 1×10^6 chick embryo cells from trypsinized primary cultures were added to 60 mm plastic Petri dishes in medium 199 plus 2 per cent tryptose phosphate broth and 2 per cent chicken serum. After 40 h of incubation at 38° C the cultures were fully confluent and 3 μ g/ml. of crystalline trypsin (Sigma) was added to the medium. At intervals the cultures were labelled with 0.25 μ Ci/ml. of ³H-thymidine for 1 h and the radioactivity insoluble in 5 per cent trichloroacetic acid was counted. The fraction of cells in mitosis was determined by microscopic count after fixation with Bouin's solution and staining with Harris's haematoxylin stain. The number of cells per dish was determined after trypsinization by counting in a Coulter electronic counter.

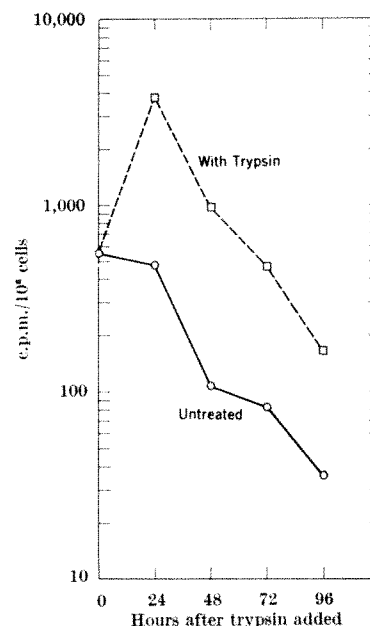


Fig. 2. Rate of incorporation of ³H-thymidine for a 4 day period after trypsin was added to the medium. The procedures were the same as in Fig. 1 except that 5×10^5 cells were plated in Scherer's medium, the cells were incubated for 72 h before treatment, and labelling was done at 24 h intervals.

continue to do so until confluence is restored³. Growth of confluent cells can also be stimulated by the addition of a macromolecular component of serum⁵. We now report that the addition of proteolytic enzymes to the medium of inhibited cells in concentrations too low to cause cell detachment stimulates rapid growth among the cells.

A concentration of 3 μ g/ml. of crystalline trypsin (Sigma) was sufficient to release cells from growth inhibition when added to the medium of confluent, growth inhibited cultures (Fig. 1). The only visible effect of this concentration of trypsin was to increase slightly the refractility of the cells. Three hours after the addition of trypsin an increase in the rate of incorporation of ³H-thymidine per cell into acid insoluble material was observed. The rise in the rate of incorporation of ³H-thymidine in the treated cultures correlated with later increases in mitotic rate and total cell number. This correlation justified the use of ³H-thymidine incorporation as an index of the rate of cell multiplication. The maximal rate of incorporation of ³H-thymidine per cell, twenty-one-fold higher than the untreated control, occurred 6-7 h after the addition of trypsin. It then decreased somewhat to a rate roughly ten-fold higher than that of the untreated cultures and this difference in rate continued until the termination of the experiment at 21 h. Autoradiographic studies show that this increased rate of incorporation in the treated cultures was the result of an increased fraction of the population incorporating ³H-thymidine rather than of an increased rate of incorporation in a constant fraction of the population (Table 1). At the time of maximal response 53 per cent of the treated cell population and only 2.9 per cent of the untreated population incorporated ³H-thymidine during the 1 h labelling period. This peak probably resulted from a partially synchronous release from inhibition. Mitosis reached a maximum in the treated cultures at 11 h. Between 8 and 20 h after the addition of trypsin, the number of cells in the treated population doubled. During the 21 h time-span of this experiment, the rate of incorporation of ³H-thymidine and mitosis in the untreated cultures remained essentially constant and only a 15 per cent increase in total cell number occurred. The presence in the medium of 3 μ g/ml. of trypsin can maintain, in some

experiments, an increased rate of ^3H -thymidine incorporation relative to untreated controls for up to 96 h (Fig. 2).

The stimulation was proportional to the concentration of trypsin up to 2 $\mu\text{g}/\text{ml}$. and was then independent of further increases in concentration up to 15 $\mu\text{g}/\text{ml}$. (Fig. 3). At higher concentrations the cells were removed from the dish. At least two other proteases, pronase and ficin, which are less specific in their activities than trypsin also release cells from growth inhibition (Table 2).

Table 1. FRACTION OF CELLS INCORPORATING ^3H -THYMIDINE AS DETERMINED BY AUTORADIOGRAPHY

Hours after addition of trypsin	0	2.5	4.5	6.5	8.5	10.5	12.5	14.5	16.5	20.5
Per cent cells labelled	2.9	10.6	23.7	32.7	53.0	30.8	23.1	20.1	23.5	16.6

Same experiment as Fig. 1. The cells were labelled for 1 h with 1 $\mu\text{Ci}/\text{ml}$. of ^3H -thymidine, fixed with Bouin's solution and covered with emulsion. Before radioactivity was counted they were stained with Harris's haematoxylin stain.

Table 2. RATE OF INCORPORATION OF ^3H -THYMIDINE INTO FICIN AND PRONASE TREATED CULTURES

Treatment	c.p.m./ 10^4 cells
Control	600
1 $\mu\text{g}/\text{ml}$. pronase	3,040
3 $\mu\text{g}/\text{ml}$. trypsin	4,180
Control	1,070
5 $\mu\text{g}/\text{ml}$. ficin	3,000
1.25 $\mu\text{g}/\text{ml}$. ficin	1,570
1.25 $\mu\text{g}/\text{ml}$. ficin	1,320
3 $\mu\text{g}/\text{ml}$. trypsin	11,400

Cultures containing 1.6×10^6 cells were prepared as in Fig. 1. After incubation for 36 h the indicated amounts of enzymes were added and ^3H -thymidine incorporation was measured 20 h later.

The rate of incorporation of ^3H -thymidine per cell was inversely proportional to the population density of cells in a culture (Fig. 4). When trypsin was present in the medium, however, the rate of incorporation was much less dependent on population density. Trypsin reduced the rate of incorporation per cell of sparse cultures (fewer than $4 \times 10^5/60$ mm dish) and increased it in dense cultures (more than $4 \times 10^5/60$ mm dish) to a rate almost independent of density.

An experiment was done to determine whether trypsin stimulates growth by acting directly on the cells or by producing a stimulatory product in the medium. Medium was first incubated with trypsin and then an excess of soybean trypsin inhibitor was added before placing the mixture on inhibited cultures. Trypsin treated medium failed to stimulate ^3H -thymidine incorporation if soybean

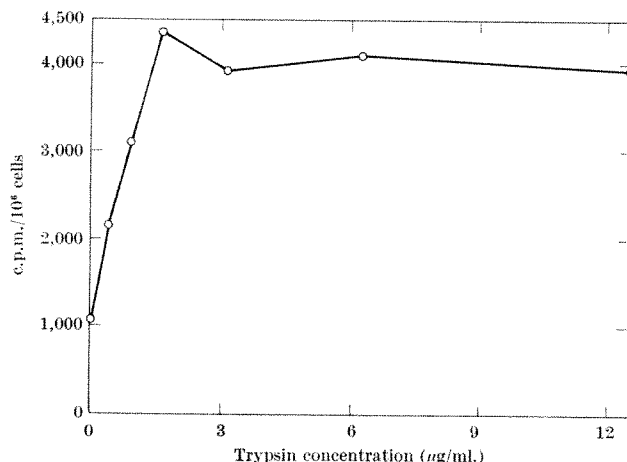


Fig. 3. Incorporation of ^3H -thymidine as a function of the concentration of trypsin in the growth medium. The procedures were the same as in Fig. 2, the trypsin was added at the indicated concentrations, and ^3H -thymidine incorporation was measured 18 h later.

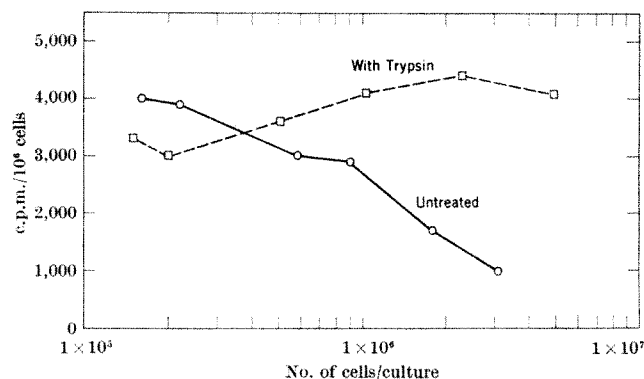


Fig. 4. Incorporation of ^3H -thymidine as a function of cell density. Cultures were prepared at six densities according to the procedures of Fig. 1. Trypsin (3 $\mu\text{g}/\text{ml}$.) was added 4 h after seeding. At 24 h after seeding, cell number was determined by trypsinization and Coulter counting and ^3H -thymidine incorporation was measured.

trypsin inhibitor was added but did so if the inhibitor was omitted (Table 3). Significantly, the inhibitor did not prevent the stimulation of cells by pronase. These results show that trypsin acts directly on the cells to stimulate their growth and that it does so through its specific proteolytic activity.

Table 3. EFFECT OF SOYBEAN TRYPSIN INHIBITOR ON GROWTH STIMULATION PRODUCED BY TRYPSIN AND PRONASE

Medium included in agar overlay	c.p.m./ 10^4 cell
Control	2,394
Pretreated with trypsin	11,029
Pretreated with pronase	4,625
Control plus inhibitor	2,290
Pretreated with trypsin plus inhibitor	2,956
Pretreated with pronase plus inhibitor	4,062

A previously described⁴ agar overlay assay was used. Medium was incubated for 30 min at 38°C at pH 7.2 with either 3 $\mu\text{g}/\text{ml}$. trypsin or 1 $\mu\text{g}/\text{ml}$. pronase and then mixed with 20 $\mu\text{g}/\text{ml}$. soybean trypsin inhibitor (Worthington) and added to the cells. 24 h later ^3H -thymidine incorporation was measured.

Because trypsin is not taken up into living cells⁷, disrupts monolayers without killing cells, and is known to alter various components of the plasma membrane⁸⁻¹⁰, its growth stimulating effect probably stems from limited proteolysis of the cell surface. The possibility that the growth stimulation results from the removal of cells from the surface of the dish was excluded by microscopic examination which showed that the number of nuclei per microscopic field did not decrease at any time after the trypsin was added.

There are two plausible but not mutually exclusive explanations for the effect of trypsin. One is that the permeability of the cells to certain growth regulating elements such as ions¹¹ or nutrients¹² is altered by the action of trypsin. The other is that trypsin inactivates surface recognition sites involved in sensing the proximity of other cells. There is no basis at present to choose between the two possibilities.

The stimulation of growth produced by proteases in culture raises the question whether these or related enzymes play a part in growth regulation in the natural state. Some findings which support this notion are the stimulation of salivary gland growth by proteases¹³ and the existence of peptidase and esterase activities in preparations of the skin, epithelial, mesenchymal and nerve growth factors¹⁴⁻¹⁶. Peptidases are particularly abundant in the periphery of many tumours where stromal and vascular stimulation occurs¹⁷, and Rous sarcoma virus transformed cells release a growth stimulating factor which has some properties suggestive of proteases⁶. A further analysis of the biological significance of proteases in growth regulation should therefore be studied further.

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Inhibition of Bone Marrow Colony Formation by Normal and Leukaemic Human Serum

A FACTOR is present in the serum and urine of mice and humans which can stimulate the formation of granulocytic and macrophage colonies by mouse bone marrow cells in agar culture¹⁻³. Levels of this colony stimulating factor (CSF) can be assayed in bone marrow cultures because of the sigmoid relationship between CSF concentration and colony numbers³. Previous studies showed that serum CSF levels were elevated in mice with various types of leukaemia^{1,4}. Although serum colony stimulating activity seemed to be high in some patients with leukaemia⁵, a direct comparison of CSF levels in normal and leukaemic human sera was prevented by an inability to detect CSF in normal human serum.

During studies on the fraction of CSF from mouse serum, it was found that a lipoprotein fraction was present in serum which could inhibit colony stimulation by CSF and thus mask the detection of CSF. Although this inhibitory material was non-dialysable, it precipitated from the serum during dialysis of the serum for 3 days against water and could subsequently be removed by centrifugation⁶. Application of this dialysis procedure to normal human sera has allowed low levels of CSF to be detected in all of 50 sera assayed, mean colony counts of ten (range 3-25) being obtained using 0.1 ml. of serum with 75,000 C57Bl bone marrow cells.

An assay system has now been developed for inhibitor levels in human sera based on the capacity of the test serum to inhibit colony formation stimulated by standard pools of highly active (dialysed) human sera from patients with renal disease. Assays were performed in 35 mm Petri dishes using 1 ml. cultures of 0.3 per cent agar in Eagle's medium containing 75,000 C57Bl bone marrow cells. The culture technique has been described in full elsewhere⁴. In the assay, 0.05 ml. of the active serum pool and 0.1 ml. of the test serum were mixed in the Petri dish before the addition of the agar-medium containing bone marrow cells. Colony counts were performed on replicate culture after incubation for 7 days at 37°C in 10 per cent CO₂ in

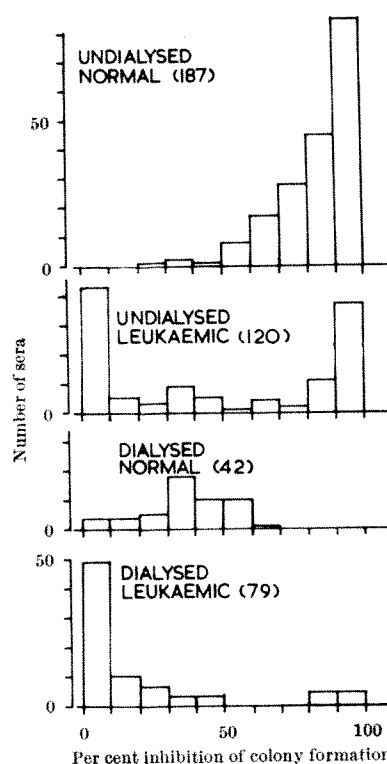


Fig. 1. Distribution of inhibitor levels in normal sera and sera from patients with acute granulocytic leukaemia, before and after dialysis. Number of sera assayed indicated in parentheses.

air, and inhibitor levels were expressed as the mean percentage reduction in colony counts compared with counts in control cultures containing the active serum alone. Control colony counts ranged from 40-75 per culture according to the active pool used.

A survey of 187 sera from healthy blood donors aged 20-65 yr revealed that 98 per cent of the sera had inhibitor levels ranging from 50-100 per cent (Fig. 1) and this was accepted as the normal range. Tests on forty-two of these sera after dialysis against water for 3 days showed that inhibitor levels had been reduced to levels ranging from 0-60 per cent and 85 per cent of the dialysed sera showed inhibitor levels below 50 per cent. Parallel assays were made on 120 sera from 38 adult patients with acute granulocytic leukaemia. Inhibitor levels in these leukaemic sera had a bimodal distribution, 46 per cent of the sera having inhibitor levels within the normal range whereas the remainder had subnormal levels and 36 per cent of the sera exhibited no detectable inhibitory activity. Dialysis of leukaemic sera reduced inhibitor levels in most, but occasional sera persisted in exhibiting 100 per cent inhibition of colony formation, a phenomenon not evident with dialysed normal serum.

Direct and indirect evidence suggests that CSF operates in the body as a regulator of granulopoiesis and macrophage formation⁷, but the biological significance of the present inhibitors is quite unknown. It is possible that the inhibitory activity observed is an *in vitro* artefact, but the inhibitors in human sera were not toxic for colony cells and did not inhibit colony formation stimulated by mouse CSF. Further work is required to determine the incidence of low inhibitor levels in various disease states and the clinical significance of fluctuations in serum inhibitor levels is being investigated in a sequential study on leukaemic patients. One possibility is that these inhibitors may operate *in vivo* to modulate, or balance, the stimulating effects of CSF on granulocyte and macrophage formation. If so, then the low inhibitor levels in acute

granulocytic leukaemia may be of significance in contributing to the abnormal granulopoiesis in these patients.

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Importance of Immunologically Competent Cells in Contact-induced Cytotoxicity

It is not yet clear whether the cytotoxicity observed when target cells and allogeneic lymphoid cells are cultured together *in vitro*^{1,2} is mediated by an immunological mechanism. Cell death has been attributed to contact with the antigenically or structurally incompatible lymphocyte cell surface rather than by any conventional immunological reaction^{2,3}. Thus any cell type carrying foreign histocompatibility antigens would be able to cause target cell death. The following experiments were carried out to test this hypothesis.

Mouse ascites tumours (BP8 of C3H origin, or Sarcoma 1 of A strain origin) were used as the source of target cells. 10^6 tumour cells were incubated for 24 h at 27°C in Hanks balanced salt solution with 10 per cent calf serum, and the medium was then changed to medium 199 with 10 per cent calf serum, and phytohaemagglutinin when BP8 target cells were used. 10×10^6 effector cells were added to the tumour cell cultures, and incubation was continued for a further 48 h. The supernatant was removed, replaced with 0.1 per cent eosin, and living (unstained) and dead (stained) cells were counted. Each combination of cells was set up in triplicate and most readings were done "blind".

80 to 85 per cent target cells were viable after incubation alone or with syngeneic lymph node cells. 50 to 55 per cent BP8 cells (Table 1) and 60 to 65 per cent Sarcoma 1 cells (Table 2) survived incubation with allogeneic or semi-

Table 2. EFFECTIVENESS OF LYMPHOID CELLS (10×10^6) FROM IMMUNOLOGICALLY INCOMPETENT MICE IN PRODUCING DEATH OF SARCOMA 1 (A STRAIN) TARGET CELLS (10^6)

Effector cell donor	Treatment of donor	Time after treatment	Per cent living cells after 48 h
—	—	—	83
Syngeneic	—	—	85
Allogeneic	—	—	62
Allogeneic	Tolerance induced with A cells	20 weeks	84
Allogeneic	Tolerance induced with A \times C3H cells	20 weeks	83
Allogeneic	Tolerance induced with CBA cells	20 weeks	64
Allogeneic	Neonatal thymectomy	8 weeks	64
Allogeneic	Neonatal sham-thymectomy	8 weeks	62
Allogeneic	ALS treated	1 week	84
Allogeneic	ALS treated	2 weeks	86
Allogeneic	ALS treated	3 weeks	64

syngeneic lymph node cells. Using Snedecor's variance ratio test these results were found to be highly significant.

Experiments with non-lymphoid effector cells yielded negative results (Table 1). Allogeneic red blood cells had no effect on the target cells, and epidermal cells (prepared by tryptic digestion of mouse tail skin) were also non-cytotoxic. Although sarcoma cells were slightly cytotoxic, this effect was also observed with syngeneic sarcoma cells and probably resulted from the development of generally toxic culture conditions, such as exhaustion of the medium by the large number of rapidly metabolizing cells. Table 1 also shows the cytotoxic ability of lymphoid effector cells. Spleen cells were competent to cause significant cytotoxicity, but to a lesser degree than lymph node cells. Thymus and bone marrow cells were both inactive. Cells from newborn mice and one week old mice did not kill target cells, but by the second week they had evidently become competent to do so.

The cytotoxic capacity of effector cells from tolerant mice is shown in Table 2. Lymphocytes from C3H mice made tolerant to the target genotype (A strain) did not kill target cells, but lymphocytes from mice made tolerant to another genotype (CBA) remained cytotoxic. Because the tolerant mice were cellular chimaeras and might have many lymphocytes of donor origin (in the former case syngeneic with target cells), chimaera tests were carried out to determine the number of donor cells. One was an *in vitro* cytotoxicity test using an anti-C3H antiserum raised in A strain mice, and the other was an *in vivo* method designed by Mitchison⁴ and modified by Brent and Gowland⁵, who used the test quantitatively. Both tests confirmed the results of Brent and Gowland⁵, only about 5 per cent of the cells being of donor origin. Later, tolerance to the target cell genotype was induced with semi-syngeneic (A \times C3H) cells, and in sufficient numbers these would be cytotoxic (Table 1); however, cells from these animals had no cytotoxic effect. It is clear therefore that the number of donor cells present cannot account for the observations, and that the host cells must have become non-reactive as a result of neonatal exposure to target cell antigens.

To avoid the complication of cellular chimaerism neonatally thymectomized mice were used as effector cell donors (Table 2). Cells from these animals did not have any cytotoxic effect, but cells from sham-thymectomized animals reacted normally.

The effects of lymph node cells from mice treated with antilymphocytic serum (ALS) are shown in Table 2, the effector cells being taken at weekly intervals after treatment. The results show that their cytotoxic capacity was at first suppressed, but that it returned within 3 weeks. In parallel *in vivo* experiments, lymph nodes were taken from mice at weekly intervals after treatment and injected into hybrid recipients which were examined for splenomegaly ten days later. It was found that cells taken 1 and 2 weeks after ALS treatment did not cause splenomegaly, whereas cells taken at week 3 were as effective as cells from untreated mice in producing splenomegaly. This indicates that the return of competence detected in the *in vivo* system corresponds to the return of the cytotoxic capacity of the cells *in vitro*.

These studies suggest that only cells capable of mounting an immunological response can cause contact-induced

Table 1. EFFECTIVENESS OF VARIOUS TYPES OF EFFECTOR CELLS (10×10^6) IN PRODUCING DEATH OF BP8 TARGET CELLS (10^6)

Effector cell donor	Age of donor	Effector cell type	Per cent living tumour cells after 48 h
—	—	—	82
Syngeneic	Adult	Lymph node	84
Allogeneic	Adult	Lymph node	54
Semi-syngeneic	Adult	Lymph node	55
Allogeneic	Adult	Erythrocytes	82
Allogeneic	Adult	Epidermal	84
Syngeneic	Adult	Sarcoma	74
Allogeneic	Adult	Sarcoma	76
Allogeneic	Adult	Spleen	62
Allogeneic	Adult	Thymus	84
Allogeneic	Adult	Bone marrow	85
Allogeneic	1 day	Spleen	85
Allogeneic	7 days	Spleen	82
Allogeneic	14 days	Spleen	56

cytotoxicity. Immunologically incompetent cells, both lymphoid and non-lymphoid, were not cytotoxic. The finding that lymphoid cells became reactive after the first week of life agrees with work by Brent and Gowland⁶, which indicated that at least some mice do not have their full complement of competent cells until after this time. The fact that lymphoid cells from mice tolerant to the target cell genotype were not cytotoxic, unlike cells from mice tolerant to another genotype, strongly suggests a specific immunological reaction occurring *in vitro*. Möller and Lapp have found⁷ that lymphoid cells from tolerant mice can destroy a monolayer of the strain to which tolerance has been induced. Maculla^{8,9} found that, in the case of some tissues, the embryonic organs possess antigens absent from adult tissue; because Möller and Lapp used embryonic tissue as a source of target cells, and induced tolerance with adult spleen cells⁷, this might explain their observations of a cytotoxic effect with lymphoid cells from the tolerant animals.

The only result indicative of a non-immunological mechanism is the cytotoxicity obtained with hybrid lymphoid cells which should be unable to react against parental strain target cells. This "hybrid effect" could be caused by mechanisms other than that proposed by Möller and Möller²; the tumours may possess "specific" or viral-induced transplantation antigens, or minor alloantigens caused by genetic divergence in the strain of origin, and F₁ hybrids may react more strongly than the parental strain because of hybrid vigour. Cudkowiec has proposed¹⁰ that all antigens present in the two parental strains may not necessarily be expressed fully in the F₁ hybrid. These possibilities would explain hybrid cytotoxicity on an immunological basis, and the effect is therefore being investigated further.

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Single Hit Aetiology of Human Minor Congenital Malformations unassociated with Major Congenital Malformations

MINOR birth defects can be defined as anatomical variants which occur at low frequency in the normal population and, as distinguished from major malformations, have no clinical or cosmetic effects *per se*. Many syndromes associated with major defects such as the trisomies and rubella embryopathy also have a high incidence of minor malformations. Conversely, almost all minor defects which have been studied clinically are known to be more frequent in individuals with major malformations. The latter types of malformations have been shown to cluster in the sense that an individual with a major defect in one

organ or system is more likely than an individual chosen at random to have a significant defect elsewhere¹. But we have found that minor malformations in individuals without major malformations do not show such a tendency to cluster but rather are independent. Furthermore, our subsequent analysis of published data of other workers which bear on this problem supports this conclusion.

Between July 7 and September 5, 1969, 202 white newborn infants in a local hospital were examined by one of us (J. J. P.) using the methods of an earlier study¹. Babies were selected only by their presence in the hospital on a weekday during that time and the availability of maternal age and birth weight from their records at the time of examination. We specifically looked for anomalies which had been classified as minor malformations by Marden *et al.* (Tables 3 and 4 in ref. 1), at Madison, Wisconsin. Sydney lines, flat nasal bridges and hyperextensible thumbs were also scored as minor defects. The decision to include these additional defects was made before the Poisson distribution was calculated. The analysis here is limited to those 200 babies in whom no major malformations were detected.

In Table 1 the distribution of minor malformations and the comparison with the expected findings on the assumption of a Poisson distribution are given. Table 2 provides a brief summary of the observations of the Albany and Madison studies.

Table 1. OBSERVED AND EXPECTED DISTRIBUTION OF MINOR ANOMALIES IN MADISON AND ALBANY STUDIES

No. of anomalies per baby	Madison†		Albany§	
	Observed*	Expected†	Observed	Expected†
0	3,713	3,723.4	121	118.9
1	574	554.8	58	61.8
2	33	41.3	17	16.1
3	2	2.1	4	2.8

* Calculated from Table 2 of the Madison study assuming three anomalies in each of the two babies listed as having three or more anomalies.

† Calculated from Poisson distribution².

‡ Goodness of fit $\chi^2 = 2.363$, $0.50 > P > 0.25$ (2 d.f.).

§ Goodness of fit $\chi^2 = 0.827$, $0.75 > P > 0.50$ (2 d.f.).

Table 2. COMPARISON OF MADISON AND ALBANY STUDIES

	Madison	Albany
Total number of babies	4,322	200
Proportion male	0.498	0.543
Total number of anomalies	646*	84 (105)
Mean number of anomalies per baby	0.149	0.420 (0.520)
Total number of babies with anomalies	609	67 (79)
Frequency of anomalous babies	0.141	0.335 (0.395)

* Calculated from Table 2 of the Madison study¹ assuming three defects in each of the two babies listed as having three or more defects.

Figures not in parentheses refer to those defects listed by the Madison study (Tables 3 and 4) which were found in this study. The number of anomalous babies, 67 in 200, is significantly different from the Madison total ($\chi^2 = 54$, $P < 0.001$). Totals in parentheses include those scored by the Madison study as well as Sydney lines, flat nasal bridges and hyperextensible thumbs.

The Poisson distribution tests the tendency of observed events to cluster. If the observations being scored occur independently and thus are the results of single hits by multiple causal events, then their distribution should agree with that predicted by the Poisson distribution. If they cluster, that is, are frequently the consequence of multiple hits by single causal events, then their distribution should deviate from the Poisson. Analysis of our data and those published after the earlier investigation reveals a close agreement with that predicted on the assumption of a single hit aetiology (Table 1). In spite of this the frequency of minor malformations was significantly higher in the Albany than in the Madison population, even when the three additional defects scored in the Albany study were excluded (Table 2). This may reflect (1) different ethnic and socio-economic structures of the populations studied; (2) differences in type and extent of teratological factors operating on the two populations, and/or (3) differences between the two studies in the diagnostic criteria for particular defects. This question is currently being investigated. It is interesting, however, that two studies carried out 6 years apart by different observers with different anomalies scored, populations studied and incidence of anomalies both revealed a Poisson

distribution. This strongly implies that the observations reflect a real human biological phenomenon.

There is one exception to the general conclusion. The Madison study apparently scored a unilateral malformation (such as a single simian line) with weight equal to a bilateral malformation. To facilitate comparison, we did the same with our own data. When we analysed individual malformations of paired structures, however, we found that if a defect was present in one of a paired structure it was more frequently present in the contralateral structure in the baby studied than in a baby chosen randomly (Table 3). This is also apparent from the Madison data in which both unilateral and bilateral incidences were presented. Thus minor malformations unassociated with major malformations do cluster, but only with regard to bilateral manifestation.

Table 3. UNILATERAL AND BILATERAL INCIDENCE OF DEFECTS ($\times 10^{-3}$)

	Madison			Albany		
	Unilateral	Unilateral ^{2*}	Bilateral	Unilateral	Unilateral ^{2*}	Bilateral
Lack of helical fold	28.9	0.84	6.2	50	2.5	25
Ear tags	0	0	2.3	26	0.6	0
Simian creases	26.6	0.70	16.7	40	1.6	30
Sydney lines	—	—	—	45	2.0	30
Clinodactyly	5.1	<0.1	4.9	5	<0.1	20
Epicanthal folds	0.9	0	3.2	5	<0.1	5

* The square of the unilateral incidence. The expected bilateral incidence assuming independence of lateral manifestation is twice the product of the incidence on either side. This cannot be calculated from the data in their present form but is less than the square of the total unilateral incidence which is given in this column. The bilateral incidences are greater than these latter figures in ten out of eleven cases in which the comparison can be made. By the sign test³ this is significant at at least the 5 per cent level.

These conclusions apply only to the malformations investigated. If we could investigate minor defects elsewhere in the body, such as variations in vascular supply and visceral anatomy, we might find that they were positively correlated with the occurrence of the more superficial anomalies studied. Furthermore, particular pairs of minor malformations may show a positive tendency to associate even though analysis of the distribution of all minor birth defects had no such general tendency. But many more data would be required adequately to investigate this question.

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In Vitro Conversion of DDT to DDD by the Intestinal Microflora of the Northern Anchovy, *Engraulis mordax*

THE tissues of many species of fish contain DDT and two of its derivatives, DDE and DDD. Fish which feed on plankton, such as the northern anchovy *Engraulis mordax*, acquire DDT residues primarily through the assimilation of food¹⁻³. The reductive dechlorination of DDT to DDE and DDD has been demonstrated in three species of salmonid fishes: Atlantic salmon^{4,5}, cutthroat trout⁶, and rainbow trout⁷. The purpose of this investigation

was to determine whether the intestinal microflora of the northern anchovy is capable of dechlorinating DDT and to elucidate the relative importance of bacteria and fungi in this process.

The intestinal contents of twenty-five adult anchovies were drained into a sterile test tube to which 15 ml. of sterile Difco nutrient broth was added. Aliquots (2 ml.) were injected into twenty sterile test tubes (24 ml. capacity) equipped with vaccine caps, and the cultures divided into four experimental groups: (i) series A was left unaltered; (ii) series B was inoculated with 100 units each of penicillin and streptomycin to suppress bacterial activity; (iii) series C was inoculated with 100 units of mycostatin to suppress fungal activity; and (iv) series D was inoculated with 100 units each of penicillin, streptomycin and mycostatin. One control was run with each series, in which nutrient broth was substituted for intestinal contents.

Each culture was inoculated with 0.05 ml. of 111 p.p.m. ¹⁴C-DDT ethanol solution and incubated anaerobically in a nitrogen atmosphere at 15° C in the dark for 6, 12, 24, 48 and 72 h. After digestion with a glacial acetic-perchloric acid mixture⁸, 4 ml. of water was added and the DDT residues extracted with 10 ml. of hexane and concentrated to 0.5 ml. in a Kuderna Danish evaporator. The DDT residues in 0.05 ml. of the concentrate were separated by descending chromatography using 2,2,4-trimethylpentane as the mobile solvent and 8 per cent 2-phenoxyethanol in anhydrous ethyl ether as the immobile solvent⁹. The chromatograms were cut into 1 cm horizontal strips and counted with a Nuclear Chicago Unilux II scintillation counter. The peaks were identified by parallel chromatography using 1/100 DDT, DDE and DDD standards and AgNO₃ as the chromogenic agent⁹.

After the extraction was completed, each culture was filtered through HA 'Millipore' filters, and the filter (particulate fraction) and filtrate (polar fraction) counted with the scintillation counter.

Table 1. RELATIVE AMOUNT (PER CENT) OF ACTIVITY RECOVERED IN EACH FRACTION AND THE TOTAL AMOUNT (d.p.m.) OF ACTIVITY RECOVERED FROM EACH CULTURE INCUBATED WITHOUT PENICILLIN, STREPTOMYCIN, AND MYCOSTATIN (GROUP A), WITH PENICILLIN AND STREPTOMYCIN (GROUP B), WITH MYCOSTATIN (GROUP C), AND WITH PENICILLIN, STREPTOMYCIN AND MYCOSTATIN (GROUP D)

Experimental group	Incubation time (h)	Per cent				Total activity (d.p.m.)
		Hexane fraction DDT	Hexane fraction DDD	Polar fraction DDE	Particulate fraction	
A-1	6	45.0	36.0	0	0.9	588,825
2	12	27.3	32.7	0	1.7	593,140
3	24	30.6	35.0	0	4.0	606,768
4	48	29.5	27.9	>0.1	2.6	605,312
5	72	25.0	54.1	>0.1	2.2	597,120
Control	72	93.9	0	0	1.0	610,694
B-1	6	52.7	27.2	0	0.7	599,228
2	12	25.5	29.0	0	1.9	580,897
3	24	49.8	22.9	0	2.3	596,178
4	48	40.9	24.8	>0.1	2.8	601,580
5	72	41.6	37.8	>0.1	1.9	598,244
Control	72	98.0	0	0	0.3	618,226
C-1	6	18.1	27.6	0	1.5	597,821
2	12	23.7	35.3	0	1.5	590,254
3	24	37.0	22.1	0	1.2	609,078
4	48	34.5	38.8	>0.1	1.6	599,890
5	72	21.3	42.7	>0.1	2.4	596,330
Control	72	81.9	0	0	1.5	596,714
D-1	6	56.2	25.8	0	0.6	613,946
2	12	76.5	16.9	0	0.9	609,300
3	24	67.6	13.6	0	0.7	592,666
4	48	48.1	34.4	0	1.5	592,985
5	72	39.2	41.2	0	1.6	597,110
Control	72	99.4	0	0	0.2	621,109

Most activity was recovered in the hexane fraction (Table 1). No ¹⁴C-DDD or DDE appeared in the controls, and the activity found in the polar and particulate fractions was considerably less than in the test cultures. All the test cultures contained high concentrations of ¹⁴C-DDD, but little or no ¹⁴C-DDE. The highest levels of labelled DDD occurred in group A in which neither bacterial nor fungal activity was suppressed, and the lowest in group D where both were suppressed. Both

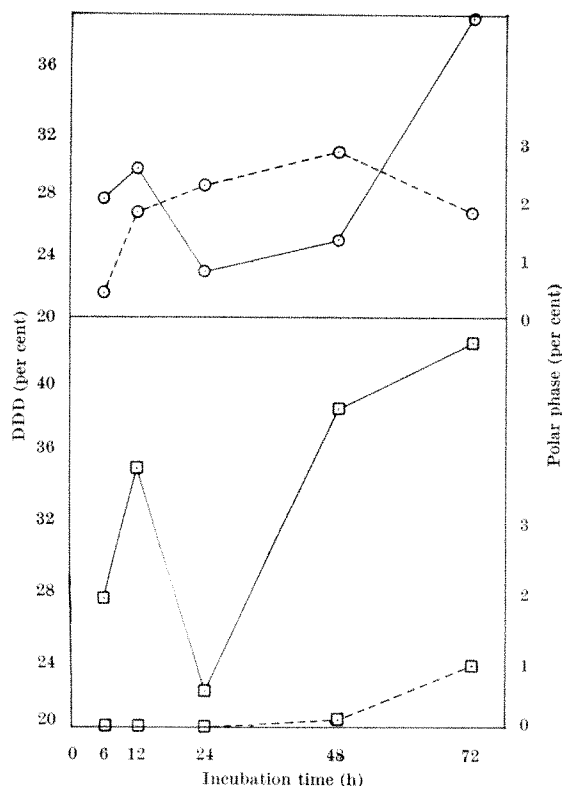


Fig. 1. Relative amount of labelled DDD (—○—) and polar fraction activity (---□---) recovered as a function of incubation time. Values were calculated after subtracting controls. Bacterial activity in group B (○) and fungal activity in group C (□) were inhibited.

fungi and bacteria converted DDT to DDD, and the efficacy of this conversion is shown by the high level of ^{14}C -DDD observed after only 6 h of incubation in group A. This rapid metabolism of DDT to DDD may have important consequences for survival because DDD is less toxic than DDT^{10,11}, and the degradation and assimilation of ingested food require several days¹².

A relatively large amount of labelled material remained in the cultures after the hexane extraction. From 18 to 53 per cent of the recovered activity was particle bound in groups A, B and C. Particle bound activity was only 6 to 18 per cent in group D where microbial activity was most inhibited. Polar phase activity was also lowest in group D where it constituted 0.6 to 1.6 per cent of the recovered activity as opposed to 0.7 to 4.0 per cent in groups A and B. This may indicate further metabolism of DDD to water soluble products such as DDA and the incorporation of labelled material into refractory organic compounds.

Some differential activity of the intestinal fungi and bacteria is suggested. In group B, where bacterial activity was suppressed, DDD levels were lower and polar phase activity higher than in group C where fungal activity was suppressed (Fig. 1). This indicates that while both bacteria and fungi metabolized DDT to DDD, the fungi were primarily responsible for further degrading DDD to a water soluble product in anaerobic conditions.

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Effect of Iron on the Bactericidal Proteins from Rabbit Polymorphonuclear Leucocytes

WHILE investigating the bactericidal action of the basic proteins released from the lysosomes of rabbit polymorphonuclear leucocytes, we found that the rate of destruction of staphylococci was influenced by the medium in which the test was carried out. When a chemically defined medium¹ (containing phosphate buffer at pH 7.4, glucose, seventeen amino-acids, thiamine, nicotinamide and salts of Mg^{2+} , Mn^{2+} , Fe^{2+} or Fe^{3+}) was used, the bactericidal action of the leucocyte extracts was markedly inhibited and in certain cases abolished. The inhibition was due to the presence of Fe^{2+} or Fe^{3+} .

Two methods were used for obtaining extracts from the lysosomes of rabbit polymorphs: (1) treatment of whole leucocytes with staphylococcal leucocidin in the presence of Ca^{2+} (refs. 2 and 3); (2) acid extraction of the lysosomal fraction obtained by sucrose gradient ultracentrifugation⁴, followed by separation of the bactericidal basic proteins (GE)⁵ on a 'QAE-Sephadex' column (personal communication from A. Griffiths and A. C. Allison) and concentration either by freeze-drying or precipitation by $(\text{NH}_4)_2\text{SO}_4$. GE at a concentration of 0.02 mg/ml. was used in all the experiments described here.

The bactericidal activity of these extracts was tested by incubating well washed organisms from an overnight culture at 37° C in the chemically defined medium previously described, but omitting any metal salts. Fig. 1 shows the bactericidal action of GE on eight strains of staphylococci and its reversal by Fe^{2+} in a concentration of M/2,000 in M/1,500 sodium citrate. Sodium citrate alone had no effect. In some strains, the bactericidal action was completely reversed by Fe^{2+} , whereas in others it was only partially reversed. This difference was not related to the ability of the organism to produce coagulase. Fig. 2 shows a similar bactericidal action of GE on two strains of *B. anthracis*. Both strains, Sterne vaccine and the virulent M36, were sensitive to GE, and the effect was reversed by Fe^{2+} . Similar results were obtained with *Strep. faecalis*, *Listeria monocytogenes*, *Ps. aeruginosa* (Fig. 3) and *E. coli*. The latter two organisms not only survived but grew rapidly in the presence of GE when Fe^{2+} was present. Haematin containing an equivalent amount of Fe was even more effective than free Fe^{2+} .

There was a relationship between the concentration of GE used and that of Fe^{2+} required to overcome its bactericidal action, suggesting a reaction between Fe^{2+} and the basic proteins. This was confirmed by adding FeSO_4 to a neutral solution of GE, when a precipitate giving a strongly positive reaction for Fe^{2+} was formed. The precipitate was soluble at pH 2.0, and after dialysis overnight at this pH lost its reaction for Fe^{2+} , and remained soluble when the pH was raised to 7.0. The protein precipitated by Fe^{2+} contained a 5–10-fold greater bactericidal specific activity than the original GE, and the protein remaining in the supernatant was inactive. Fe^{2+} -precipitation has accordingly been used as a step in the purification of GE.

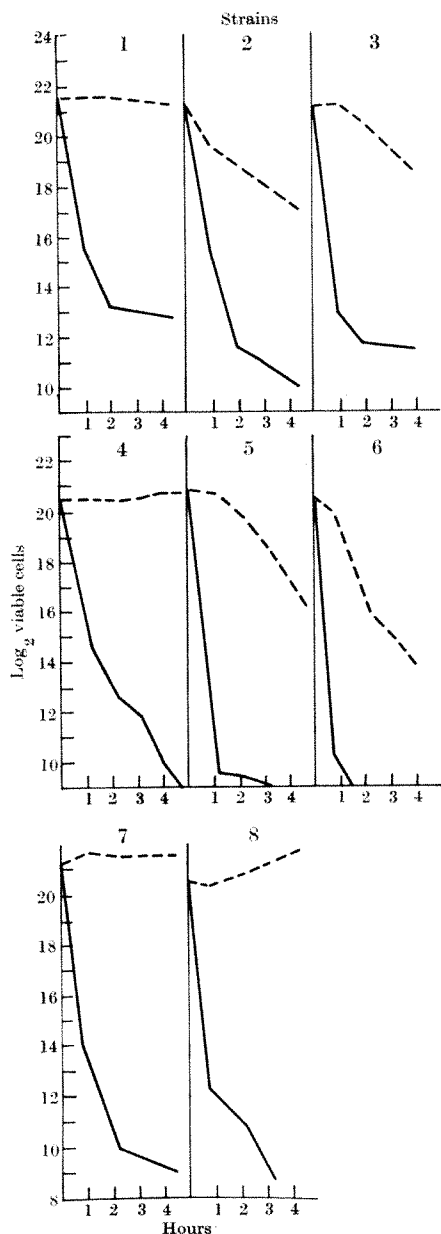


Fig. 1. The reversal by Fe^{2+} of the bactericidal action of basic proteins from rabbit polymorphs on staphylococci ($10^6/\text{ml.}$). Strains 1-4, 7, 8 coagulase positive; strains 5, 6 coagulase negative. —, Without Fe^{2+} ; ----, with $\text{M}/2,000 \text{ Fe}^{2+}$.

Although from these results it is clear that Fe^{2+} reacts with and inhibits the action of the bactericidal basic proteins from leucocytes, it may also inhibit other bactericidal proteins in leucocyte lysosomes. The effect of $\text{M}/2,000 \text{ Fe}^{2+}$ on the bactericidal action of lysozyme at a concentration of 0.02 mg/ml. was tested on a sensitive strain of *Staph. epidermidis* and shown to be negative. Fe^{2+} was also tested in the myeloperoxidase- H_2O_2 system^{6,7} in the conditions described by Klebanoff⁷ using *E. coli* and *Staph. epidermidis*. In neither case was the bactericidal action by $\text{M}/2,000 \text{ Fe}^{2+}$.

The effect of Fe in increasing the virulence of bacteria has received much attention⁸⁻¹¹. In a discussion of their observations on the effect of Fe in enhancing the virulence of *E. coli* and *Past. septica*, and in reversing the protective effect of antisera, Bullen and Rogers¹¹ interpret their results as due to a reversal of the bacteriostatic action of normal and immune serum by saturation of the serum transferrin with Fe. It is postulated that the combined effect of transferrin, β_2 and γ -globulin in the serum

interferes with the Fe metabolism of the organism, and the addition of Fe restores the balance.

Using mice injected intraperitoneally with a sublethal dose of *E. coli* with and without Fe^{2+} , we have confirmed the enhancing effect of Fe on virulence (Table 1). We have also demonstrated a profound effect of Fe^{2+} (Table 2) and haematin in enhancing the virulence of *Staph. aureus* and of Fe^{2+} in enhancing that of *B. anthracis*. These Gram positive organisms are not subject to the bacteriostatic or bactericidal action of antibody and complement. We have not been able to demonstrate a bactericidal or bacteriostatic action of normal rabbit serum on *Staph. aureus* or *Staph. epidermidis* provided the serum is

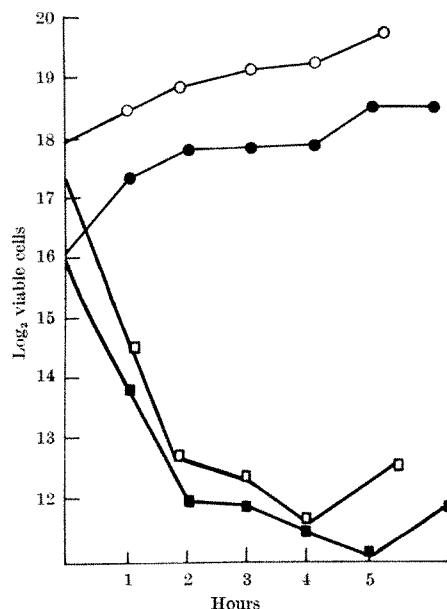


Fig. 2. The reversal by Fe^{2+} of the bactericidal action of basic proteins from rabbit polymorphs on *B. anthracis* (two strains). Conditions as in Fig. 1. Sterne strain: ■, GE alone; ●, GE + Fe. M36: □, GE alone; ○, GE + Fe.

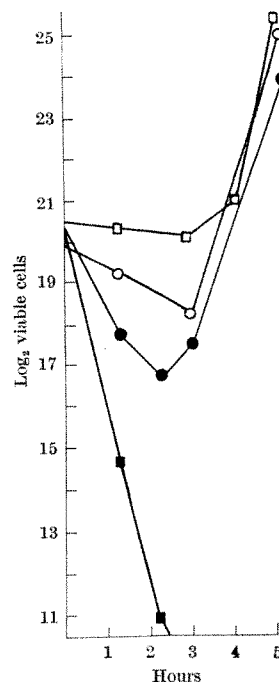


Fig. 3. The reversal by Fe^{2+} of the bactericidal action of basic proteins from rabbit polymorphs on *Ps. aeruginosa*. Conditions as in Fig. 1. ●, Control; ○, + Fe; ■, GE alone; □, GE + Fe.

Table 1. EFFECT OF Fe^{2+} ON THE VIRULENCE OF *E. coli* IN MICE

Group	Number of mice	Concentration of Fe^{2+}	Deaths in 18 h
1	12	M/700	11
2	12	M/1,400	10
3	12	M/2,800	8
4	12	0	0

10^7 washed cells of *E. coli* from an overnight culture were injected intraperitoneally with the stated concentration of FeSO_4 in equimolar sodium citrate.

Table 2. EFFECT OF Fe^{2+} ON THE VIRULENCE OF *Staph. aureus* IN MICE

Group	No. of mice	Concentration of Fe^{2+}	Deaths	Total deaths
			Day: 1 2 3 ... 14	
1	12	M/160	8 3 0 ... 0	11
2	12	M/320	4 1 0 ... 1	6
3	12	M/640	3 2 1 ... 0	6
4	12	0	0 0 0 ... 0	0

Conditions as in Table 1 using 10^8 washed cells of *Staph. aureus*.

obtained from clotted plasma¹². It therefore seems unlikely that the system described by Bullen and Rogers plays a part in increasing the virulence of these organisms. Although serum transferrin may not be concerned, however, Masson, Heremans and Schonne¹³ have recently reported the presence of the iron-binding protein lactoferrin in neutrophil leucocytes, which may act similarly. Masson, Heremans, Prignot and Wauters¹⁴ using lactoferrin from bronchial mucus showed that it was bacteriostatic for *Staph. epidermidis*.

A sample of apo-lactoferrin from human milk was tested for bactericidal action on staphylococci and for its reversal by Fe^{2+} in the same conditions as in our previous tests. A bactericidal action was obtained only when very large doses (1 mg/ml.) were used (Fig. 4), and the effect was not reversed by Fe^{2+} . It is therefore clear that the bactericidal action of GE is not attributable to lactoferrin.

Preliminary results from work on the intracellular killing of bacteria by whole polymorphs have shown that Fe^{2+} has a marked effect in protecting staphylococci from destruction, although phagocytosis is not affected by the concentration of Fe^{2+} used.

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Viable Algae in House Dust

HUMAN allergic reactions to algae have been documented¹, but so far these disorders have been recognized only after exposure to algae-infested waters. A number of investigators have suggested, however, that air-borne algae constitute a source of respiratory hypersensitivity reactions in susceptible individuals²⁻⁴. Viable cultures of algae were obtained from atmospheric samples by van Overeem in 1937⁵. More recently, Gregory *et al.*⁶, Schlichting⁷ and Brown *et al.*³ confirmed and extended these findings. Air-borne dissemination of algae was suspected as an explanation for the high incidence of positive skin reactions, reaginic antibodies, and positive provocative responses obtained in atopic patients tested with aqueous extracts of various species of green algae⁴. It seemed that one likely source of air-borne algae would be ordinary house dust and we now report the results of our investigation of this possibility.

Aliquots (0.1 g) of house dust collected from various indoor localities in Cincinnati, Ohio, between February and May 1969 were inoculated into two culture media: Bristol⁸ and modified Chu No. 10 (ref. 9). Viable algae were cultured from all the dust samples taken from forty-one homes. In these culture conditions the most frequently observed organisms were *Chlorella*, *Chlorococcum*, *Schizothrix*, *Planktosphaeria*, *Chlamydomonas*, and *Anabaena* (Table 1). Samples from three commercial producers of house dust allergenic extract also revealed viable algae (Table 2). In general, the algal organisms found in house dust used in the manufacture of commercial allergenic products belonged to the same genera that were frequently observed in dust cultures collected from individual homes.

Quantitative data were obtained for commercially derived dust samples by plating 0.01 g of dust, diluted in

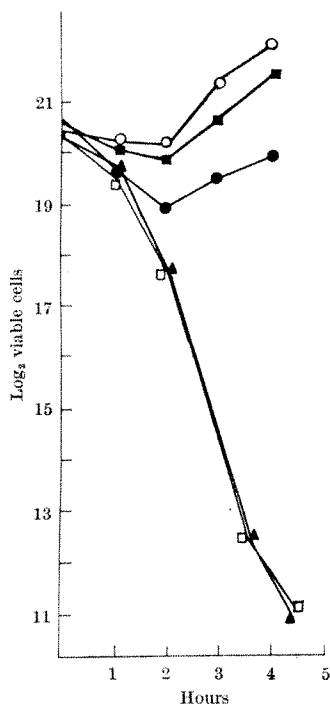


Fig. 4. The effect of apo-lactoferrin on *Staph. epidermidis*. Conditions as in Fig. 1. ●, Control; ■, lactoferrin alone (0.2 mg/ml.); ○, lactoferrin (0.2 mg/ml.) + Fe^{2+} ; ▲, lactoferrin alone (1 mg/ml.); ▲, lactoferrin (1 mg/ml.) + Fe^{2+} .

Table 1. DISTRIBUTION OF ALGAE IN DUST SAMPLES FROM FORTY-ONE HOMES

Algal genera	No. of dust samples	Algal genera	No. of dust samples
<i>Anabaena</i>	14	<i>Nitzschia</i>	1
<i>Chlamydomonas</i>	14	<i>Oocystis</i>	2
<i>Chlorella</i>	27	<i>Oscillatoria</i>	6
<i>Chlorococcum</i>	24	<i>Palmella</i>	1
<i>Dictyosphaeria</i>	1	<i>Palmelloccoccus</i>	3
<i>Fragilaria</i>	2	<i>Planktosphaeria</i>	16
<i>Gomposphaeria</i>	1	<i>Scenedesmus</i>	3
<i>Kentrosphaeria</i>	1	<i>Schizothrix</i>	21
<i>Microcoleus</i>	1	<i>Stichococcus</i>	3

Tris buffer, on Bristol and modified Chu No. 10 agar plates. These plates were prepared using a basal layer of 1.5 per cent agar which was overlaid with an inoculum layer of 0.5 per cent agar. The average algal counts per g for Purex, Center and Hollister-Stier Laboratories were 175, 240 and 1,250, respectively. (These represent minimal values for the competitive inhibition and colonization characteristics of many algae undoubtedly affect the efficiency of plating.) Allergenic extracts for human studies were prepared from organisms isolated from two homes and from a commercial sample supplied by Hollister-Stier Laboratories. *Chlorococcum* and *Chlorella* species were from home samples while a second *Chlorella* species was obtained from the commercial source. These members of the order Chlorococcales are commonly encountered in sampling for air, soil and water-borne algae⁴. All cultures were purified by repetitive subculturing on plates.

Table 2. COMPOSITE LIST OF ALGAE ISOLATED FROM HOUSE DUST USED IN MANUFACTURE OF COMMERCIAL ALLERGENIC PRODUCTS

Commercial source	Algae
Purex Co.	<i>Chlorella</i> <i>Anabaena</i>
Center Labs.	<i>Chlorococcum</i> <i>Stichococcus</i> <i>Schizothrix</i> <i>Chlorella</i>
Hollister-Stier Labs.	<i>Schizothrix</i> <i>Chlorella</i>

Eighty-four patients undergoing allergic evaluation for suspected respiratory allergy were first skin tested intracutaneously with 0.02 ml. of 1:10,000 dilutions of four commercial house dust extracts (Purex, Hollister-Stier, Center and Endo). Only reactions consisting of erythema and a circular weal measuring at least 0.5 cm in diameter were considered positive. If skin tests were negative at this dilution, patients were retested at 1:1,000 dilutions. Thirty-seven patients reacted to 1:10,000 dilutions of one or more of the commercial house dust extracts and forty-one patients showed positive reactions to 1:1,000 dilutions of one or more of the same extracts. At the same time skin tests were also performed with allergenic extracts prepared from the three house dust algal isolates. Of the thirty-seven patients who reacted to 1:10,000 dilutions of the various commercial house dust extracts, twenty-two displayed positive reactions to one or more of the algal extracts diluted 1:10,000 and five reacted to the same extracts diluted 1:1,000. In the group of forty-one patients who showed reactions to dust extracts diluted 1:1,000, only one reacted positively to a 1:10,000 dilution of the algal extracts whereas twenty-one reacted positively to algal extracts diluted 1:1,000. Most of the algal positive patients (70 per cent) reacted to all four dust extracts, the highest correlation (87 per cent) being observed in patients reacting to an extract prepared from the Hollister-Stier *Chlorella* isolate. Of the dust-positive patients who gave negative reactions to the algal extracts, only 35 per cent reacted to all four dust extracts. No skin reactivity could be elicited with any of the algal extracts in patients showing negative skin test responses to house dust extracts. This correlation was not attributed to lack of skin reactivity, for these patients gave positive reactions to other common allergens (fungi and pollens of ragweed, grass and trees).

Of the 84 patients tested, 58 per cent showed positive responses to one or more algal allergens, consistent with our previous results⁴. Cross reactivity between the algal allergens differed somewhat in this study, however. Previously⁴ no significant differences or similarities were observed between skin test reactivity of *Chlorella* and *Chlorococcum* allergens. Here we observed that 69 per cent of patients showing positive tests to the extracted *Chlorella* isolate from Hollister-Stier also reacted to the other two allergenic isolates, one of which was a *Chlorococcum* species.

House dust, a highly complex material with many known allergenic components¹⁰, also seems to be a likely source of human exposure and sensitization to many varieties of algae, which may give rise to clinical allergic problems.

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Asymmetrical Distribution of the Potential Difference in the Toad Lens

THE electrical p.d. across the plasma membrane of cells may be either uniformly distributed around its surface or, as in composite epithelial membranes such as the amphibian skin and urinary bladder, asymmetrically distributed. In the former type of cell, ion transport takes place from the inside to the outside of the cell. In the latter, ions move between the bathing solutions across the entire cell.

An electrical p.d. of about 60 mV (negative inside the lens) has been measured between the inside of the amphibian lens and its external bathing medium¹⁻³. It has generally been assumed, but not proved, that this p.d. is uniformly distributed around the entire surface of the lens. This feature, together, with the high K⁺ and low Na⁺ and Cl⁻ concentration in the lens, has prompted the comparison with a giant cell⁴. The observations which we describe here indicate that this is not valid in the lens of the toad *Bufo marinus*.

We have measured the Na⁺, K⁺ and Cl⁻ content of the ocular fluids and the lens. The concentrations (mequiv/kg water) in the proportions vitreous : lens : aqueous are 111 : 31 : 117 for Na⁺; 3.8 : 82 : 2.3 for K⁺; 108 : 26 : 101 for Cl⁻ (means of ten). The ionic gradients across the toad lens thus conform to the usual pattern. Storage of the toad lens for 24 h at 5° C results in an accumulation of Na⁺ and Cl⁻ and a loss of K⁺. We have also immersed the isolated toad lens in modified Ringer solution⁵ and inserted a microelectrode (filled with 3 M KCl) in it through the capsular wall and found—as in the frog lens—an electrical p.d. of 59 ± 2.5 mV, with the outside surface positive.

Other experiments show, however, that this electrical p.d. is not uniformly distributed. This became apparent to us after carrying out two types of experiments. Initially we suspended the lens in a glass "cradle" con-

nected with rubber tubing to a reservoir filled with Ringer solution (Fig. 1a). The exposed face of the lens was kept moist by dripping Ringer solution. When Ringer agar bridges were placed so as to record the p.d. across the whole lens from both its surfaces, a mean electrical p.d. of 23 ± 1.7 mV was observed (anterior face positive).

The second method involved inserting the lens into a piece of glass tubing joined at each end to a chamber which contained circulating Ringer solution (Fig. 1b). When the lens was fixed in such a "divided chamber" an electrical p.d. of 25 ± 2.9 mV (anterior face positive) was recorded. The short circuit current (s.c.c.), amounted to 8 ± 0.8 μ A per 0.28 cm² cross-sectional area of the lens (28.5 μ A/cm²). The p.d. and s.c.c. were stable for several hours. In s.c.c. conditions, unidirectional fluxes of Na⁺ and Cl⁻ using ²²Na and ³⁶Cl were measured. Sodium fluxes were equivalent to: anterior to posterior 1.3 ± 0.4 μ A, posterior to anterior 2.4 ± 0.5 μ A; Cl fluxes: anterior to posterior 2.53 ± 0.16 μ A; posterior to anterior 2.28 ± 0.21 μ A. Clearly the net Na or Cl fluxes or both cannot explain the total s.c.c. Also, considering the high concentration of K⁺ in the lens and that the inside of the lens is negative with respect to both bathing solutions, it is very unlikely that an electrogenic K⁺ pump could be responsible for the p.d. and the s.c.c. It is possible that ions other than Na⁺, K⁺ or Cl⁻ are being actively transported across the whole lens, namely, H⁺ or HCO₃⁻; that a non-electrogenic Na⁺:K⁺ pump is maintaining the Na⁺ and K⁺ gradients or that organic ions are being produced inside the lens and a differential permeability between the anterior and posterior surfaces produces the p.d. across the whole lens.

Finally, another series of experiments were conducted to elucidate the contribution of each surface to the p.d. Microelectrodes were inserted laterally into the middle of a lens mounted in the "divided chamber" (Fig. 1b). In this way the p.d. across both the anterior and posterior surfaces could be individually studied, and the effect of ionic replacement, in either bathing solution, on each p.d. measured. The normal p.d. across the anterior surface was found to be 57 ± 4.2 mV whereas the p.d. across the posterior surface was 31 ± 3.8 mV (inside in both cases negative). The p.d. across the whole lens was 26 ± 2.3 mV.

Table 1. EFFECTS OF IONIC CHANGES ON THE p.d. ACROSS THE ANTERIOR AND POSTERIOR SIDES OF THE TOAD LENS

Bathing solution	Change in p.d. (mV)	
	Posterior	Anterior
Cl ⁻ -free posterior side	0	0
Cl ⁻ -free anterior side	0	+4
Na ⁺ -free posterior side	+6	0
Na ⁺ -free anterior side	0	-7
K ⁺ , 75; Na ⁺ , 31; Cl ⁻ , 33 posterior side (mequiv/l.)	-6	0
K ⁺ , 75; Na ⁺ , 31; Cl ⁻ , 33 anterior side (mequiv/l.)	-7	-27

The p.d. on the two sides of the lens behaved differently when the ionic compositions of their bathing solutions were altered (Table 1). Omission of Cl⁻ had little effect. Na⁺-free media caused a small decline in p.d. at the anterior face and a similar increase at the posterior side. Elevation of K⁺ caused a rapid decline of the p.d. at the anterior side to a stable value of 20 mV but had little effect posteriorly. All the changes were reversible and the individual effects on each side were added when ionic changes were made simultaneously in both solutions.

These results clearly indicate that (a) the lens is not a symmetrical organ but a polarized one, the electrical

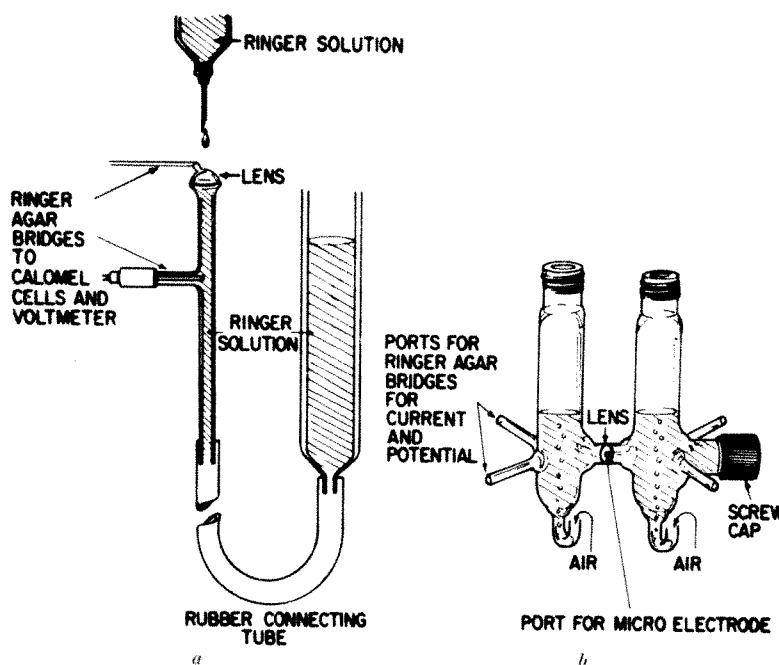


Fig. 1. a, "Cradle" system for supporting the toad lens *in vitro*; b, "divided chamber" system for studying the electrical properties and permeability of each side of the lens. The p.d. and s.c.c. are recorded from Ringer-agar bridges inserted through the ports in each chamber. A microelectrode can be inserted through a small hole in the tubing connecting the two chambers and into the lens so that recordings of the p.d. can be made across each side of the tissue.

properties of its anterior and posterior surfaces being different; (b) Cl⁻ is relatively unimportant for the maintenance of p.d. at either surface; (c) removal of Na⁺ has an opposite effect on each surface suggesting that Na⁺ is necessary in the anterior bathing solution for the maintenance of at least part of the p.d. Its effect on the anterior side is opposite to what we would expect if Na⁺ moves at that level only by diffusion; (d) the anterior surface is more sensitive to depolarization by K⁺ than the posterior.

Our results suggest that at least partially the ionic gradients and the p.d. found between the lens and surrounding humours are the result of the operation of a Na⁺ pump located at the anterior surface level that moves Na⁺ from inside the lens to the outside solution. The entire lens is surrounded by a capsule. Underneath the capsule—on the anterior side only—a single layer of epithelial cells is present. It seems likely that these cells contribute to the asymmetrical electrical properties of the lens.

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Role of the Perineural Sheath of Peripheral Nerve on Fluxes of L-Glutamate *in vitro*

THE possibility that L-glutamate might be the excitatory transmitter released by the central terminals of sensory afferent fibres has prompted some studies on its release in peripheral nerves^{1,2}, but there have been no systematic studies of the resting fluxes of glutamate and it is not known whether the perineural sheath is a barrier to the movements of this substance.

Short segments of sheathed and desheathed frog (*Rana catesbiana*) sciatic nerves or "bags" made from isolated sheaths were used in this study. For studying fluxes in whole nerves, uniformly ¹⁴C-labelled L-glutamic acid (188 mCi/mmol, New England Nuclear Corp.) was added to the frog Ringer solution to give a final concentration of 22 μ M, 4.08 μ Ci/ml., and for studies on isolated sheaths the concentrations of tracer substances were: ¹⁴C-glutamate, 5.4 μ M; ¹⁴C-sucrose, 1.011 mM (135 mCi/mmol, Calbiochem); ³HOH, 75 mM (25 mCi/g, New England Nuclear Corp.); ³⁶Cl⁻, 3.6 mM (10.2 mCi/g, as NaCl, Radiochemical Centre, Amersham). Radioactivity was monitored by liquid scintillation counting and metabolism was checked using column or paper chromatography. To study the initial influxes, nerves were incubated in radioactive medium at 4°, 17° and 22° C for periods of 30 s to 30 min and to characterize the "loading process" (for

Table 1. RATE CONSTANTS OF EFFLUX OF RADIOACTIVITY FROM FROG SCIATIC NERVES BATHED IN INACTIVE RINGER SOLUTION

Preparation	Soaking time at 4° C (h)	Rate constants ($\times 10^4$) [†] K_s (s ⁻¹)	K_f (s ⁻¹)
Sheathed*	19-70	0.94 \pm 0.14 (6)	31.2 \pm 2.1 (6)
Desheathed*	19-26	1.43 \pm 0.35 (14)	29.4 \pm 1.5 (5)

* In one nerve in each of these groups, efflux was studied at 4° C; all others were at 22° C.

[†] Means \pm s.e.; numbers of nerves in parentheses. Rate constants were determined from the slopes of regression lines obtained by the method of least squares. K_s , rate constant (first order) for the slow component of efflux; K_f , rate constant (first order) for the fast component (see Fig. 1).

Table 2. RATE OF DIFFUSION OUT OF "BAGS" PREPARED FROM ISOLATED NERVE SHEATHS FILLED WITH SOLUTIONS OF LABELLED SUBSTANCES

Substance	Apparent D (cm ² s ⁻¹)	Free D (cm ² s ⁻¹)	Free/apparent
¹⁴ C-glutamate	2.2 $\times 10^{-8}$ 2.8 $\times 10^{-8}$	7.1 $\times 10^{-8}$ (from ref. 7)	285
¹⁴ C-sucrose	0.7 $\times 10^{-8}$ 1.1 $\times 10^{-8}$	5.0 $\times 10^{-8}$ (ref. 8)	553
³ HOH	1.8 $\times 10^{-8}$ 2.7 $\times 10^{-8}$	2.4 $\times 10^{-8}$ (ref. 9)	11
³⁶ Cl ⁻	1.9 $\times 10^{-8}$ 2.2 $\times 10^{-8}$	1.8 $\times 10^{-8}$ (ref. 10)	869

nerves used in efflux studies) nerves were soaked for longer periods.

A very rapid exponential accumulation of glutamate occurred within the first 5 min of immersion in the active medium, presumably because of diffusion, which was followed by a constant rate of influx for the next 25 min. The mean values for three to six nerves (for each point) were plotted for the influx which occurred at 5, 10 and 30 min and regression lines were calculated for these points by a method of least squares. Influxes (M_1) were calculated using a modification of the equation of Keynes³, according

to which, $M_1 = \frac{dY_1}{dt} \cdot \frac{C_0}{Y_0}$, where $\frac{Y_0}{C_0}$ is the external specific

radioactivity and $\frac{dY_1}{dt}$ is the regression coefficient (slope),

and were found to be 2.6, 3.7 and 5.9 pmoles/g.s in desheathed, and 1.6, 1.4 and 0.7 pmoles/g.s in sheathed nerves at 4°, 17° and 22° C, respectively. This indicates that the sheath is indeed a barrier to this temperature-dependent process.

In Fig. 1 are shown representative plots of the rates of efflux of ¹⁴C from sheathed and desheathed companion nerves at 22° C after these had been soaked in labelled medium for 19 h at 4° C. By calculating "apparent" diffusion coefficients⁴ (data not shown) and rate constants (Table 1) from these plots, it became evident that an effect of the sheath on the efflux of glutamate could not be shown by this method; the reasons for this are likely to be related to the adsorption of labelled medium to the sheath itself and to the catabolism of glutamate by the nerves. With the "bags" made from inverted nerve sheaths which were filled with Ringer solutions containing labelled substances, however, the above-mentioned difficulties were overcome, that is, a negligible catabolism of glutamate occurred, for there were no cells or axons present and there was no radioactive medium present on the outer surface of the sheath. Table 2 shows that with this method the sheath can also be shown to be a barrier to the outward diffusion of glutamate.

From these results, it is clear that the sheath is indeed a barrier to the fluxes of glutamate, but that this effect cannot be shown in whole nerves because serious errors arise from the adsorption and catabolism of glutamate.

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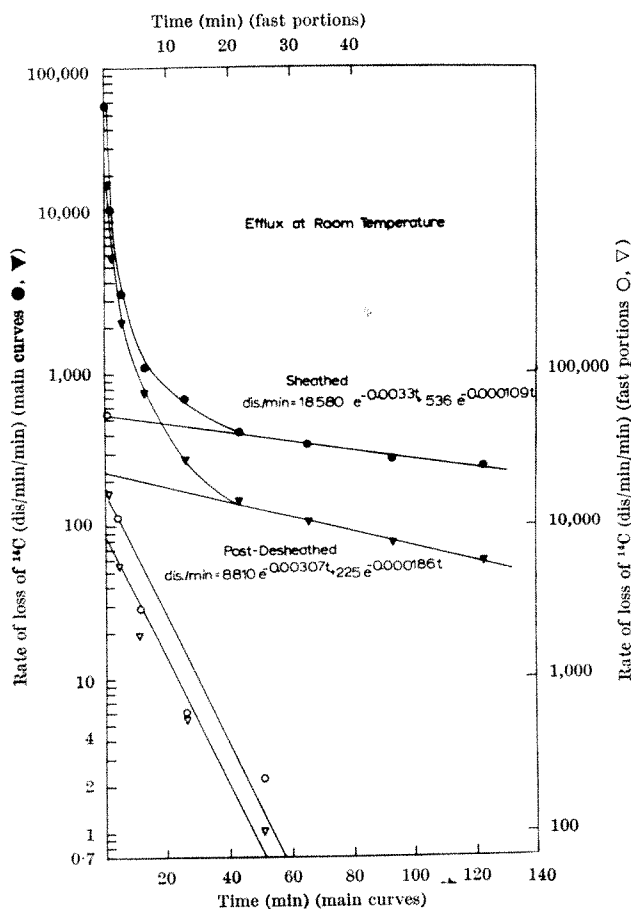


Fig. 1. Semilogarithmic plots of rates of loss of radioactivity from sheathed (●) and "post-desheathed" (▼) companion frog nerves at 22° C. ○ and ▽, Initial components of the main curves, obtained by subtraction of extrapolated slow components. The main curves and fast portions have different scales on both coordinates. Exponential equations for these processes are shown with rate constants in s⁻¹; t is time in s. Details of the method of plotting have been described previously⁴. "Post-desheathed" means that this particular nerve was stripped of its epi-plus-perineural sheath after soaking in labelled Ringer solution. Both nerves were soaked for 19 h at 4° C in Ringer solution containing 22 μ M labelled glutamate.

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Odour Discrimination: "Sex Pheromone Specialists" in the Olfactory Lobe of the Cockroach

THERE has been widespread interest in the natural sex attractant of the American cockroach, *Periplaneta americana* (L.), although its structure is unknown¹⁻⁵. The recording of the summated receptor potentials (electro-antennogram) from the *Periplaneta* showed that the sex pheromone elicited electrical responses not only in the antennae of the male but also in those of the female⁶. We have tested the responses in the olfactory centre with single units recording in the lobe, and proved that specialized neurones for the prepurified sex pheromone do exist in the olfactory lobe of both sexes.

The materials, operative procedures and recording system used in the present experiments were the same as those described earlier^{7,8}. Quantitative stimulation was brought about by a current of air passing over a piece of filter paper which was impregnated with a known amount of the substance to be tested. The substance was mounted in a small glass bottle on the air flow system in such a way that it could be easily exchanged. The prepurified sex pheromone used was extracted by the same procedure as in the previous experiment⁸.

Fig. 1 shows an example of the single cell (unit) responses of the adult male cockroach to the prepurified sex pheromone. The greater the concentration, the higher the frequency of impulses accompanying the negative slow potential which might be well explained in terms of the excitatory postsynaptic potential of the synaptic membrane. This unit also occasionally responded with a weak increase of impulse discharge to several other compounds such as cycloheptanone, cyclopentanone, geraniol, ethylene glycol, methyl ethyl ketone, *trans*-2-hexenol, acetic acid and acetone. Creosote and clove oil occasionally elicit

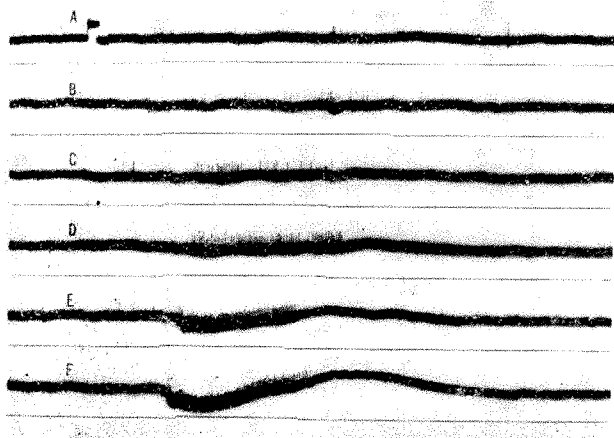


Fig. 1. The discharge of impulses of the sex pheromone specific neurone from the olfactory lobe in response to a series of papers holding prepurified sex pheromone of increasing concentration (from B to F). Before stimulation in A (control), a rectangular calibration pulse of 0.5 mV (positive with reference to the ground) was applied. Film speed, 5 cm/s. Recording was made with a longer time constant (0.3 s).

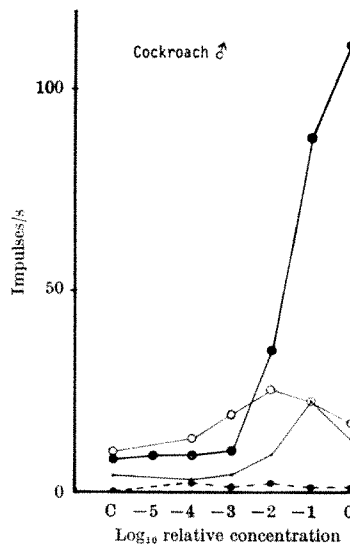


Fig. 2. Impulse frequency at different concentrations of prepurified sex pheromone (●-●), methyl ethyl ketone (○-○), cycloheptanone (●-○), and *n*-butyric acid (●-○) during the first second of response. The curve (●-●) is the mean value of nine measurements of three units in each three individuals. The other three curves represent the impulse number of one measurement of the same unit demonstrated in Fig. 1. 0 in the abscissa denotes the original concentration of each compound; -1, -2, -3, -4 and -5 denote the 10, 100, 1,000, 10,000 and 100,000-fold dilution of the original concentration, respectively; C is control (air without odour).

weak inhibition of the spontaneous discharge of this neurone. On the other hand, the unit failed to respond to twenty-eight other compounds tested.

The reaction spectra among these units found so far showed considerable overlap or exactly the same spectra for a great variety of compounds. Some are responsive only to the prepurified sex pheromone. All, however, showed very great sensitivity to the prepurified sex pheromone, compared with other effective compounds as shown in Fig. 2. We should therefore like to call these cells which respond specifically to the sex pheromone the "sex pheromone specialists".

Whether the *Periplaneta* actually uses these particular neurones (sex pheromone specialists) to inform the higher centre of the sex pheromone remains to be seen, however, because females of this species also have the particular neurones ("sex pheromone specialists") which showed great specificity and sensitivity to the prepurified sex pheromone produced by other females of the same species.

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Tentacle Responses of the Sea Anemone *Anthopleura xanthogrammica* to Ultraviolet and Visible Radiations

WE have observed that exposure of anemones to ultraviolet and visible radiations can elicit two specific responses, flexion and retraction of the tentacles. A detailed investigation revealed that these responses were readily differentiated by their spectral region of maximum sensitivity.

The sea anemone *Anthopleura xanthogrammica* was adapted to the dark for 1 h and exposed at the oral disk to determine the responses of tentacles to several wavelengths from 240 nm to 600 nm at various dose rates and exposure durations. Radiations were incident at the surface of the test organism in a field size of approximately 1.75 cm by 2.5 cm. Background illumination of the anemone by a low-level red light made it possible for a dark-adapted observer to carry out the observations.

A Bausch and Lomb high-intensity diffraction grating monochromator, with a 150 W high pressure xenon arc lamp system, provided radiations at the specified wavelengths. Separate diffraction gratings, an ultraviolet (2,700 grooves/mm) grating and a visible (1,350 grooves/mm) grating were used for the spectral regions to increase resolution. The dose rate at each wavelength was measured with a YSI-Kettering radiometer equipped with a special collecting cone. Bimetallix quartz-bonded interference filters of neutral density were used to change the dose rate over several log units.

Because the same dose rate was not attainable at all wavelengths, it was necessary, in a few instances, to adjust both dose rate and exposure duration to achieve a specific radiation dose. This was done only if prior tests of the exposure variables showed that a reciprocal relationship existed between dose rate and exposure duration for evoking the response. Using the ultraviolet spectral grating, responses were tested over the wavelength range of 248–400 nm at dose rates ranging from 0.09×10^3 erg/cm²/s to 2.99×10^3 erg/cm²/s. With the visible spectral grating, responses were tested over the wavelength range from 350–600 nm at dose rates from 0.12×10^3 erg/cm²/s to 27.2×10^3 erg/cm²/s.

It was found that exposure to wavelengths of 340–600 nm induced a flexion of tentacles, without reduction in length and with the point of flexure near the base of the tentacle. In flexion, the extended tentacle changed its relationship to the oral disk from a nearly perpendicular position to a horizontal one. Flexion was always toward the rim of the oral disk and was independent of the direction of the incident radiation beam.

Radiations of 248–400 nm induced a retraction of tentacles. During retraction, the tentacles withdrew from their normally extended position and showed constriction of the apical and mesial tentacle regions. Both flexion and retraction responses were observed among tentacles exposed in the spectral region of 340–400 nm, but were still distinguishable as two separate responses.

The minimum effective (threshold) dose (ED) for each response at each wavelength was determined by more than sixty tests performed on twenty-eight anemones, so that the relative efficiency of the wavelengths used could be compared in an action spectrum. For tentacle flexion a series of test exposures, in which the dose was changed by small increments, was made around the oral disk of each anemone. The threshold (ED) was designated as the average between a dose causing flexion and the next lower dose increment that failed to produce flexion. In the case of retraction, the duration of continuous exposure, required to elicit retraction at a given dose rate, was specified as the effective dose. This was more convenient because the low dose rates available in the

ultraviolet region meant that much longer exposures were needed.

Action spectra for flexion and retraction were based on the relative efficiency of a wavelength in producing a response as compared with a reference wavelength. This is a modification of the method outlined by Jagger¹ to measure inactivation cross-sections. The relative efficiency was calculated from the formula

$$\text{ED (reference wavelength)} \times \frac{\text{nm (test wavelength)}}{\text{nm (reference wavelength)}}$$

The action spectrum for tentacle flexion (Fig. 1), derived from relative efficiency data, shows us that radiation at 500 nm is most efficient in producing a flexion response, and that the efficiency falls off rapidly to one-third at 450 nm and one-quarter at 400 nm and 550 nm. In the ultraviolet range, the efficiency increases from 400 nm to 360 nm, and decreases rapidly below 360 nm. No tentacle flexion was observed at wavelengths below 340 nm, even at high dose rates. Experiments by North and Pantin² on bending responses of the column of *Metridium* showed a similar maximum sensitivity to light at about 490–520 nm, but no peak of sensitivity at 360 nm.

Fig. 2 indicates that there is nearly uniform low effectiveness in producing retraction in *Anthopleura xanthogrammica* by radiation of 400–320 nm. A sharp rise in efficiency occurs below 320 nm with a peak at 280 nm, declining rapidly to less than one-quarter at 248 nm.

With respect to the distribution of reacting tentacles, those of the two inner cycles showed less sensitivity to visible radiations than tentacles of the outer cycles. At 450 nm, 7 per cent of the central tentacles and 88 per cent of the peripheral tentacles responded by flexion, while at 500 nm the percentages were 8 per cent and 90 per cent respectively. The more centrally located tentacles, however, showed little difference in retraction response from peripheral tentacles when exposed to ultraviolet radiations at 280 and 360 nm.

An analysis of the photic responses of *Anthopleura xanthogrammica* indicates that sensitivity is greatest for areas of most abundant sensory and nervous elements³. Tentacle flexion (Fig. 1) might be interpreted tentatively as a reaction mediated by photosensitive cells, whether they be specialized sensory cells or epithelio-muscular cells. So far, however, no specific photoreceptors or their pigments have been identified in the anemones.

The retraction response, with a maximum sensitivity at 280 nm, suggests absorption of radiation by substances

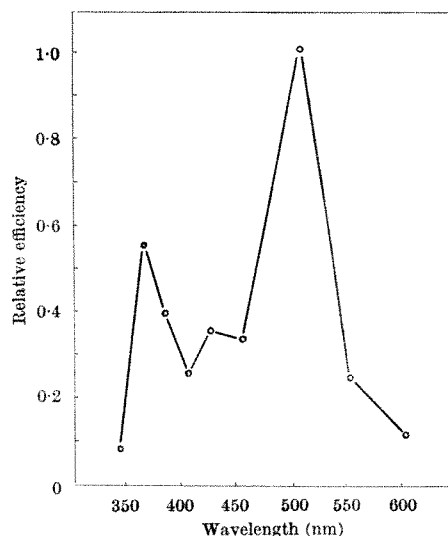


Fig. 1. An action spectrum derived for tentacle flexion in *Anthopleura xanthogrammica*.

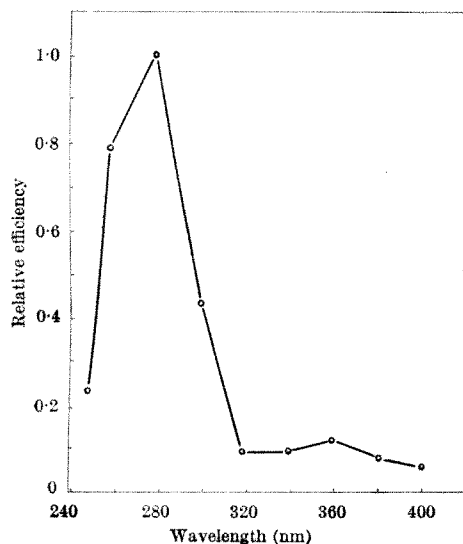


Fig. 2. An action spectrum derived for tentacle retraction in *Anthopleura xanthogrammica*.

other than typical receptor pigments, perhaps by proteins and nucleic acids. While certain insects with highly organized photoreceptor systems have been found to have a well defined response to the ultraviolet region of the spectrum⁴ and to ionizing radiation⁵, the present study seems to be the first identification of behavioural reactions to electromagnetic radiations in the ultraviolet spectral range in the phylum below the arthropods.

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Micro-injection of ¹⁴C-Sucrose into Single Living Sieve Tubes of *Heracleum*

We have made use of micro-cannulation—a new and potentially useful technique for plant physiology—by inserting glass micropipettes of 2–4 μ m tip diameter into mature living sieve tubes of *Heracleum sphondylium*. We used a micromanipulator¹ under a microscope with a Zeiss $\times 40$ water immersion objective. The technique in its present state of development may disturb the sieve element and so alter normal translocation, but it has potential advantages over the use of aphid stylets^{2,3}, for the glass is impermeable to water, the tips can be located precisely *in situ* and injection of solutes as well as withdrawal of sap are possible.

Our method was made possible by the direct observation of dancing "marker" particles which characterize functioning sieve tubes in *Heracleum*⁴. A strand of phloem 0.5 mm in diameter was isolated from xylem and parenchyma so that the strand was left attached to the plant at each end, but displaced from the living petiole. We located a suitable sieve tube under the microscope and inserted the micropipette so that the glass tip could clearly be seen in

the lumen in relation to sieve plates and marker particles. If the particles remained in the lumen near the glass tip, sparse in number and vigorous in action, damage was assumed to be minimal⁴. Because the sieve tube was under pressure, sap would usually force its way through an empty micropipette. For micro-injection we used a hydraulic system of mineral oil with the micropipette tip filled with tracer solution. The tip was inserted in the selected sieve tube, the solution was injected for 10–15 s, the tip was withdrawn and the strand was left intact for a given number of seconds. The strand was then excised at each side of the coverglass and at the basal and apical junctions with the petiole. Basal and apical sections were frozen on a sheet of solid CO₂ and further excised into sections 1–2 cm long. The tracer in each section was counted in a scintillation spectrometer for total periods exceeding 25 min. Counts were corrected for quenching and background.

In the first experiments, 0.2–0.5 μ l. of 0.3 M ¹⁴C-sucrose containing about 10⁴ d.p.m. were injected. After 5 min less than 1/100 of the radioactivity was detected within 8 cm of the origin in either direction. Most of it had disappeared. A sensitive Geiger counter placed 6 cm downstream from the microscope, however, registered several pulses of twice background in the first 2 min after injection. We therefore reduced the time between injection and excision and lengthened the isolated strand; ¹⁴C was then recovered both upstream and down, but localized in discrete sections of the strand—the shorter the time, the closer to the micropipette. Apparently injection induced a movement of sucrose in both directions away from the origin at 250–450 cm h⁻¹.

About twenty-five short-time injection experiments have been carried out so far during two summers. In twenty of these we have recovered peaks of tracer localized at rather irregular intervals with almost no counts in between (Figs. 1 and 2) and usually in both directions from the origin. To reduce possible damage in a sieve element due to injection pressure we have used as little as 2.0 $\times 10^{-3}$ μ l. (equivalent to a 6 mm length of tube containing perhaps twenty sieve elements). We still obtained tracer pulses with small injections and the distance moved in 2 min was about the same as with larger amounts. To see whether the first injection pulse had damaged the transport mechanism we have injected two 10 s pulses 60 s apart. Fig. 2B shows the result of such an experiment: the second pulse also appeared to move as readily as the first. We have also injected ⁴²KCl mixed with the

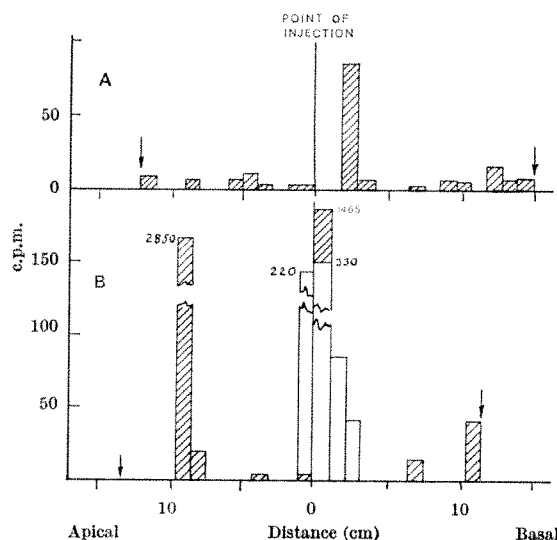


Fig. 1. Histogram showing the distribution of ¹⁴C-sucrose (hatched columns) and ⁴²K (white columns) along phloem strands of *Heracleum* 2 min after injection into a single sieve tube. Counts per minute are shown only when significantly different from background; patterns of distribution are not unusual. A, Single injection of 7 $\times 10^{-3}$ μ l., single isotope; B, single injection of 3 $\times 10^{-3}$ μ l., double isotope.

^{14}C -sucrose to see whether ^{42}K and ^{14}C travel together: as in Figs. 1B and 2B they do not seem to do so (five experiments). We have injected fluorescent dye into a sieve tube to investigate whether the dye moved in one or more sieve tubes: it seemed to move bidirectionally in a single sieve tube without appreciable lateral spreading in the first minute after injection. We have withdrawn samples from the sieve tube lumen for electron microscope study: they contain strands about 60–190 Å diameter, presumably of protein microfilaments⁵.

We have investigated the question of whether the strands we prepared can transport sucrose without injection. Dissected strands were allowed to touch a small drop of ^{14}C -sucrose + tritiated water (^3HHO) for 2 or 3 min and then excised, frozen and sectioned as before. The tracer travelled considerably further in 3 min (Fig. 3) than in 2 min. Clearly the tracer moved rapidly outward from the origin in both directions with the log c.p.m.—distance profile usually found in petioles⁶. But several types of tracer movement are evident: slow, medium and fast pulses of tracer were found in both directions to a distance from the origin which suggested speeds of 400 cm h^{-1} upwards and 280–400 cm h^{-1} downwards. In this type of experiment tracer must first be loaded into the translocation path before transport: a random loading plus transport would then give the results we obtained, but the front of the movement may indicate the distribution sum of pulse-like movements in or on individual sieve tubes. The distances traversed by the leading pulses in this experiment were similar to those from injection experiments lasting the same time. We conclude that in the displaced strands a bidirectional sucrose transport mechanism operates when the centre is the source.

The failure of ^{42}K to move far from the point of micro-injection is surprising, for it is well known that K is in the phloem sap³ and can be transported. One possibility is that the movement of K was prevented by a partial (Fig. 1B) or complete (Fig. 2B) blocking of mass flow by slime plugs at sieve plates, while transport of ^{14}C continued by an alternate mechanism. We suggest that sucrose travels on or within the microfilaments which, we think, normally traverses both sieve tube lumen and the plate pores. Thus if pressure surges from injection or strand excision causes the microfilaments to snap and form slime, irregular accumulations of ^{14}C would result. But whenever the disruption of microfilaments is not great, we suggest that sucrose is loaded onto this protenacious material and conducted in waves past sieve plates. We think that gentle injection tends to give wider bands of ^{14}C (Figs. 1A and 2A) than does high pressure injection

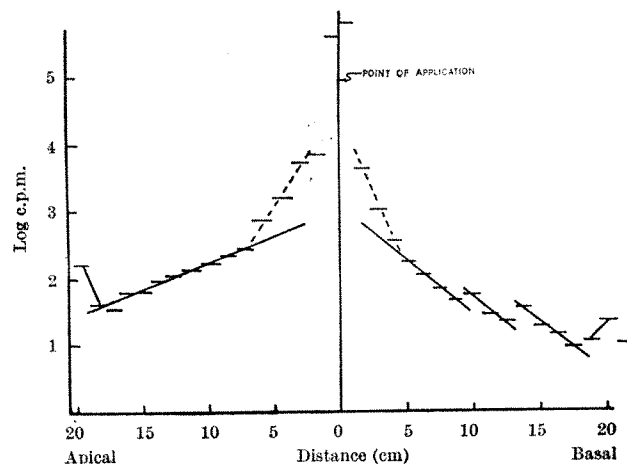


Fig. 3. Tracer profiles along an isolated phloem strand of *Heracleum* 3 min after the application of 0.3 M ^{14}C -sucrose in ^3HHO . Note the two different slopes to the log c.p.m.—distance profiles and the tracer pulses at 20 cm, apical and basal. The isotopes were counted together in this experiment because of the degree of local quenching of ^{14}C .

(Figs. 1B and 2B), but attribute the separation of tracers and irregularities to the slime plugging as against any loading-conducting properties of the microfilaments. Alternatively, the tracers might have been conducted through intercellular spaces.

We conclude that micro-injection into a single sieve tube is feasible. In our system we find evidence that two translocation mechanisms operate together, a slow mass flow for K and a much faster one for sucrose involving waves or pulses.

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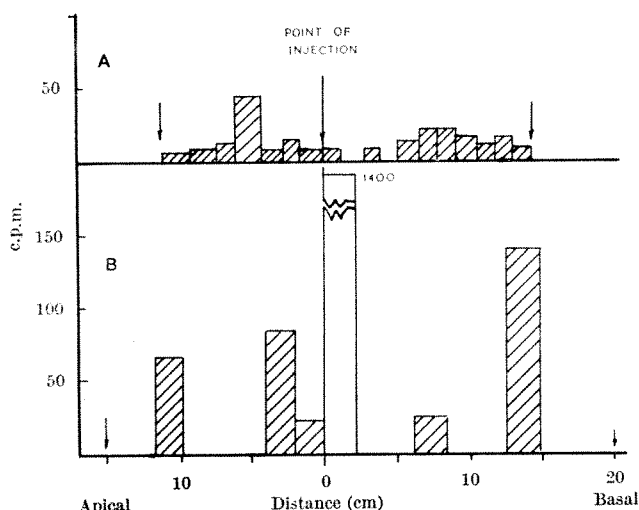


Fig. 2. As Fig. 1, A, Single injection of $5.0 \times 10^{-3} \mu\text{l}$, single isotope; pattern of distribution unusual; B, double injection of $4.0 \times 10^{-3} \mu\text{l}$, in two pulses 60 s apart, double isotope.

Effects of Inhibitors on Light-stimulated Synthesis in Radish Hypocotyl

THE effects of the inhibitors, D-threo-chloramphenicol and cycloheximide, on protein synthesis have been studied in several systems. In tissues of green plants, differential effects have been thought to be due to a preferential inhibition by chloramphenicol of protein synthesis mediated by 70S ribosomes as found in chloroplasts, while cycloheximide inhibits syntheses mediated by 80S cytoplasmic ribosomes¹⁻³ to a greater extent. We wish to report the effects of the two inhibitors on light-stimulated formation of anthocyanins and chlorophyll and on protein composition during the growth of excised radish hypocotyls.

Radish seeds (variety 'White Icicle') were germinated in the dark at 25° C for 48 h. The roots were carefully excised at the limit of the root hair zone and the testas were removed. The hypocotyls, with cotyledons attached, were incubated in distilled water (ten hypocotyls/10 ml.) and shaken continuously (100 cycles/min) at 25° C in either complete darkness or light (800 foot candles). In these conditions, the length of a hypocotyl increases consider-

ably from 5 to 21 mm (dark) or to 10 mm (light) in 48 h, entirely as a result of cell expansion.

Fig. 1 shows that cycloheximide, in a concentration greater than 10^{-7} g/ml., clearly inhibited anthocyanin synthesis to a greater extent than it affects chlorophyll formation, which is only markedly reduced by 2×10^{-6} g/ml. Chloramphenicol similarly inhibited the synthesis of both pigments in concentrations of 10^{-6} g/ml., but chlorophyll formation in the light was much more sensitive to this inhibitor— 3×10^{-6} g/ml. caused 50 per cent inhibition, while it required 3×10^{-5} g/ml. to have the same effect on anthocyanin formation.

A young radish hypocotyl contains considerable protein reserves, in the form of protein bodies, which disappear during growth, the total protein content of the hypocotyl remaining unchanged. The densitometer tracings in Fig. 2 show that proteins from unexpanded hypocotyls ("0 h" after germination for 48 h) contain a major component of mobility (relative to the mobility of the tracker dye—bromophenol blue) of 0.145. There are also some minor components of lower mobility and a diffuse series of components with a mobility of 0.15–0.60. During growth in the light for 48 h the protein component of mobility 0.145 (reserve protein) decreases in quantity; while a new sharply defined component of mobility 0.09 appears, and there is a general increase in the quantity of the protein components of mobility 0.15–0.60. The sharply defined band of mobility 0.09 has the same mobility as fraction I protein obtained from rapidly isolated radish chloroplasts, and a measurable quantity is only formed during growth in the light. Table 1 shows that the inhibitors were remarkably selective in our experiments, chlor-

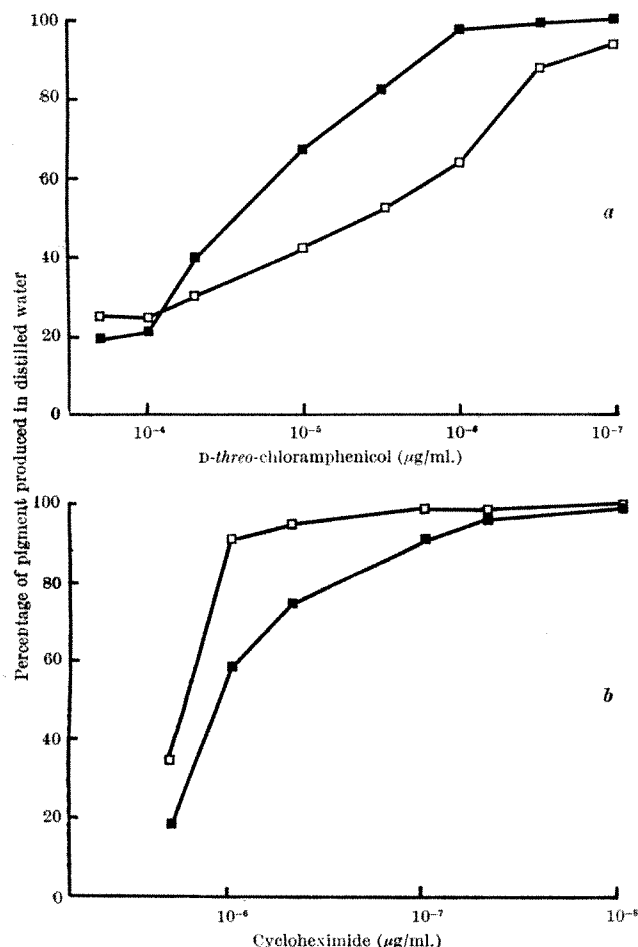


Fig. 1. Effect of D-threo-chloramphenicol (a) and cycloheximide (b) on the formation of anthocyanin (■, extracted with 1 per cent HCl in methanol, A_{540} nm) and chlorophyll a + b (□, extracted with 80 per cent acetone⁴).

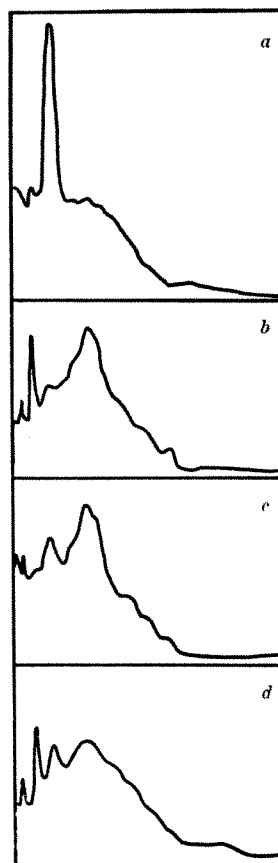


Fig. 2. Densitometer tracings of acrylamide gels stained with Coomassie blue, showing distribution of soluble proteins from hypocotyls cultured in the light for (a) 0 h; (b) 48 h in water of 50 μg/ml. of L-threo-chloramphenicol; (c) 48 h in 50 μg/ml. of D-threo-chloramphenicol; (d) 48 h in 2 μg/ml. of cycloheximide. Proteins were obtained by grinding hypocotyls in two volumes of 50 mM Tris; 0.2 mM EDTA; 10 mM MgCl₂; 5 mM reduced glutathione; filtering through nylon mesh (56 μm apertures; centrifuging at 102,600g, and subjecting the supernatant to electrophoresis on vertical slabs of 7.5 per cent polyacrylamide, pH 8.9 (ref. 6).

amphenicol completely inhibiting the formation of the protein component with a mobility of 0.09. Furthermore, this effect was only produced by the D-isomer; L-threo-chloramphenicol had no effect on the light-stimulated synthesis of fraction I protein, or on the synthesis of chlorophyll in hypocotyls or cotyledons grown in the light. This is a much clearer distinction between the D and L-isomer than that shown by MacDonald *et al.*⁵. Cycloheximide had little effect on the quantities of protein in the slow-moving bands, but caused a 25 per cent inhibition of the broad series of proteins of mobility 0.15–0.60.

These results confirm earlier reports that 1 mg/ml. of chloramphenicol inhibited the light-stimulated formation of chloroplast enzymes in *Euglena*, while cycloheximide had a smaller but variable effect³. Ingle⁷ found similar inhibitory effects of both cycloheximide (0.5–1.0 μg/ml.) and chloramphenicol (100–1,000 μg/ml.) on RNA synthesis in radish cotyledons during greening in the light, but a less clear cut difference in the effect of cycloheximide on the synthesis of chloroplast and cytoplasmic ribosomal RNA. During growth of the exised hypocotyl, no uptake of nutrients is required and a selective effect on uptake

Table 1. EFFECTS OF INHIBITORS ON FORMATION OF PROTEIN

Protein band (mobility relative to tracker dye)	Soluble protein, μg/hypocotyl* Culture conditions and duration			
	0 h dark	48 h light (water)	48 h light with CAP	48 h light with CH
0-0.08	2.3	2.6	0	2.0
0.09	0	4.5	0	4.1
0.149	36.0	3.2	8.3	9.6
0.15-0.60	111.8	140.0	141.8	133.7

CAP, D-threo-chloramphenicol, 50 μg/ml.; CH, cycloheximide, 2 μg/ml.

* Data obtained from areas under peaks on densitometer tracing.

due to interference with oxidative phosphorylation is unlikely to be involved. There is no evidence of uncoupling of oxidative phosphorylation by these inhibitors in this tissue, the effect on oxygen uptake being extremely slight. The continued use of chloramphenicol in concentrations of about 50 µg/ml. in an attempt to eliminate or reduce bacterial contamination in metabolic studies using plant tissues is clearly inadvisable, unless it is known that the inhibitor has no effect on tissue metabolism, particularly RNA and protein synthesis.

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Fixation of New Mutants

THE concept of permanent, inherited changes in proteins being brought about by the random fixation of neutral mutations has been advocated^{1,2} and criticized³ under the inappropriate heading of non-Darwinian evolution, a name better kept for other more general uses⁴.

Much of the discussion has depended on a simple result pointed out by Kimura⁵. In a population of N individuals, the chance of fixation of a new neutral mutation is $1/2N$. If the rate of mutation to such neutral variants is u per locus per gamete per generation, then if there are m loci in the population, the mean number of neutral mutants fixed per generation is $2Nmu$. $1/2N = mu$. That is, the rate per generation of fixation of neutral mutations is equal to their rate of production per gamete.

Kimura^{5,6} and King and Jukes¹ have derived rates of amino-acid substitution in proteins of approximately 18.4×10^{-10} changes per residue per year for eutherian mammals. From this, by making assumptions about the amount of mammalian DNA which is involved in structural genes, and about the average generation time in mammals, one can obtain a value as high as two neutral mutations fixed in any mammalian population in any generation. King and Jukes¹ have criticized Kimura's derivation of this value on the grounds that if all 4×10^9 base pairs of DNA in the haploid mammalian genome were involved in coding for polypeptides, there would be about 10^6 loci, and clearly with a mutation rate to lethals of 10^5 per locus, this means that an enormous number of lethal mutations must be occurring. It does not seem to have been noticed that an argument akin to Kimura's, cited earlier, leads to the conclusion that very large numbers of deleterious mutants must be fixed in each generation, if population size is small enough. It is well known^{7,8} that the chance of fixation of a new mutant with an advantage of s relative to the mean is

$$p_1 = \frac{1 - e^{-2s}}{1 - e^{-4Ns}}$$

and for a mutant with a relative disadvantage of t , the chance of fixation is

$$p_2 = \frac{e^{2t} - 1}{e^{4Nt} - 1}$$

Accordingly, with m loci and mutation rates u_1 and u_2 to appropriate alleles, the mean numbers fixed will be $2Nu_1mp_1$ and $2Nu_2mp_2$. Table 1 shows numerical values for the three cases. The value 10^{-5} was chosen for u as being of the generally accepted order of magnitude for deleterious mutation rates, and the values 10^{-6} and 10^{-7} were chosen as rates for neutral and advantageous mutations because these are clearly less likely events.

Table 1. MEAN NUMBERS OF MUTANTS FIXED PER GENERATION IN A POPULATION OF N INDIVIDUALS WITH MUTATION RATE u TO ALLELES WITH A SELECTIVE ADVANTAGE (OR DISADVANTAGE) OF s

N	m	There are m loci in the haploid genome			Mean number of mutants fixed per generation
		u	s		
100	10^4	10^{-5}	-0.001		0.081
		10^{-5}	-0.01		0.007
		10^{-6}	0.0		0.010
		10^{-7}	0.01		0.004
	10^6	10^{-5}	-0.001		8.141
		10^{-5}	-0.01		0.754
		10^{-6}	0.0		1.000
		10^{-7}	0.01		0.403
1,000	10^4	10^{-5}	-0.001		0.007
		10^{-5}	-0.01		0.000
		10^{-6}	0.0		0.010
		10^{-7}	0.01		0.040
	10^6	10^{-5}	-0.001		0.747
		10^{-5}	-0.01		0.000
		10^{-6}	0.0		1.000
		10^{-7}	0.01		3.960

This argument seems to suggest that small population size allows a greater rate of chance fixation of deleterious alleles than Haldane⁹ and Kimura and Crow¹⁰ felt possible for advantageous alleles. The result for advantageous alleles is even more extreme. Sved¹¹ and others¹²⁻¹⁴ have, however, shown that high rates of fixation of advantageous alleles are possible, given different assumptions about the mode of action of natural selection, so that it does seem likely that, as Richmond³ and Clarke¹⁵ have concluded, the reality of "non-Darwinian evolution" has yet to be demonstrated.

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Lee Wave Hypothesis for the Initial Pattern of Spread during the 1967-68 Foot and Mouth Epizootic

THE extensive and explosive initial spread of the 1967-68 foot and mouth disease epizootic in the English Midlands has been a subject of much speculation¹⁻⁴. After the first

outbreak was confirmed at Bryn farm on October 25 (day 1), some fifty-nine further outbreaks were confirmed between days 4 and 10 in a fan shaped area stretching north-east towards Chester, a distance of 56 km (see Fig. 1). The Ministry of Agriculture has argued that, because no human or mechanical links could be established between Bryn farm and subsequent outbreaks, many of these outbreaks were probably caused by the imported frozen lamb that was thought to be responsible for the Bryn farm outbreak¹. The only evidence for the view of the Ministry was that the lamb had been distributed near some of the outbreaks, and because the lamb had also been distributed extensively in other parts of England and Wales since August it seems very unlikely that many outbreaks should suddenly occur within a specific area and that they should occur approximately one incubation period after the Bryn farm outbreak.

Fig. 1 illustrates an alternative argument that Bryn farm (or Bryn farm together with neighbouring farms that might have been emitting virus at the same time) could have been the sole source and that most of the subsequent outbreaks were the result of airborne spread from Bryn farm. While the possibility of airborne spread has been confirmed for other periods during this epizootic and for other epizootics²⁻⁴, my attempts to model the spread of the disease⁴ have shown that the magnitude of the initial long range spread was far greater than in any other instance of long range spread during the remainder of the epizootic. Hence, if airborne spread was responsible for initial spread in the 1967-68 epizootic, then it was operating in a dangerously efficient manner.

Subsequent investigation revealed three curious features of the spatial pattern of initial outbreaks: the clustering of the outbreaks into three distinct clusters (see Fig. 1); the regular spacing of these clusters at 18-20 km intervals; and the fact that outbreaks in the downwind clusters occurred within the time of one incubation period after the Bryn farm outbreak. These features suggested that an atmospheric phenomenon known as lee waves may have helped the initial spread. Here I attempt to illustrate how this could have happened.

Bryn farm is located at the base of a valley ridge beside the Cheshire Plain. From October 15 to 26 the wind blew from the south-west at approximately right angles to this ridge. Fig. 2, a profile drawn through Bryn farm at an azimuth of 215°, makes these relationships clear. Given this situation, then in certain upper air conditions air flowing over a ridge or hill will be forced into vertical oscillation with a downwind configuration of the streamlines like that shown in Fig. 2. This is the phenomenon of lee waves.

It is known that the formation of lee waves can influence surface winds below the wave troughs⁵ and that material can be carried aloft and deposited downwind under the influence of the waves⁶. In a similar way lee waves could have transported virus particles from the Bryn farm area out over the Cheshire Plain. A trough over the source of virus, by enhancing up-currents, may have formed an unusually effective mechanism for carrying virus particles aloft. Once aloft, each "air parcel" is carried along the streamline pattern. Reports by glider pilots emphasize that flow in lee waves is often remarkably smooth⁷, so it seems possible that an air parcel will remain intact with a volumetric concentration of virus similar to the concentration at source. A particular air parcel might then be brought to ground level at the next trough and its virus content might be deposited or inhaled in the

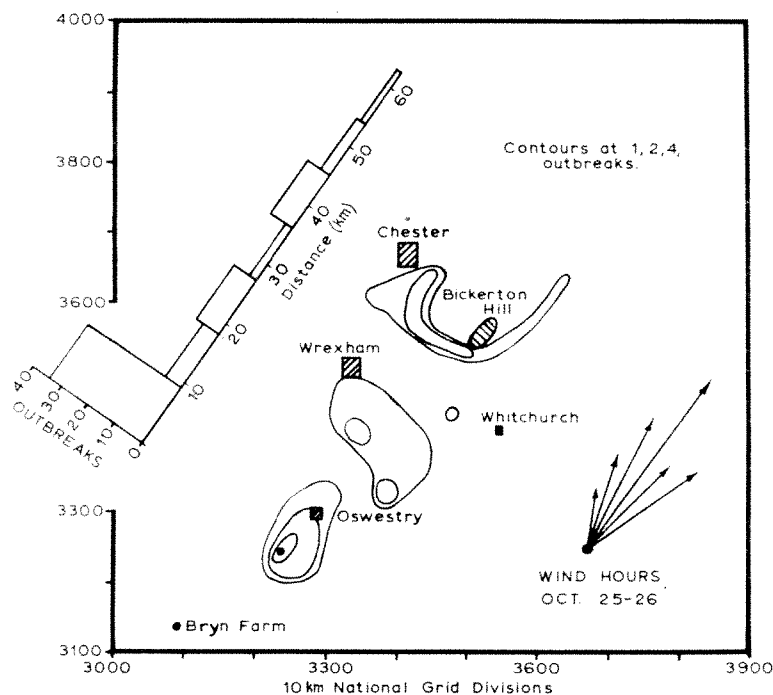


Fig. 1. Outbreaks for days 1-10.

area immediately downwind of the trough where the winds become lighter. The remainder of the air parcel might then be carried up and on to the next trough. Such a hypothesis is especially attractive (it explains how virus from a single source could cause infections at a long range downwind), but if it is to be proven it must be shown that lee waves did occur on October 25 and/or October 26; that if they did occur they had a wavelength of approximately 18-20 km, the spacing of the clusters in Fig. 1; and that the crests and troughs occurred in the positions suggested by Fig. 2 so that pickup at source and downwind deposition could be explained.

Unfortunately, it seems that no meteorological observations were made in this area at the time, so the lee wave hypothesis cannot be directly proved or disproved. Theoretical grounds have therefore been used to estimate the occurrence and essential characteristics of these lee waves.

Table 1. CONDITIONS SUITABLE FOR LEE WAVES

Condition	Camborne upper air measurements							
	October: 24	October: 25	October: 26	October: 27	October: 24	October: 25	October: 26	October: 27
1. Marked stability at lower and/or middle altitudes — indicated by isothermal layer or temperature inversion	+	+	+	+	+	+	/	/
2. Wind speed increasing rapidly with height	-	-	+	+	+	/	-	+
3. Little variability in wind direction with height	-	-	+	+	+	+	-	-
4. High wind speed at hill crest greater than 20 knots	-	-	+	+	-	-	-	-
5. Frontal system in vicinity (Oswestry)	-	-	+	+	-	-	-	-

+, means condition satisfied; /, means condition marginally satisfied; -, means condition not satisfied.

Table 1 ranks the more important of the qualitative conditions associated with the formation of lee waves⁸ and indicates that October 25 and 26 were likely days for lee waves to form. Scorer⁹ has shown that, with the assumptions of frictionless, steady, laminar and isentropic flow over small ridges, waves are only possible when

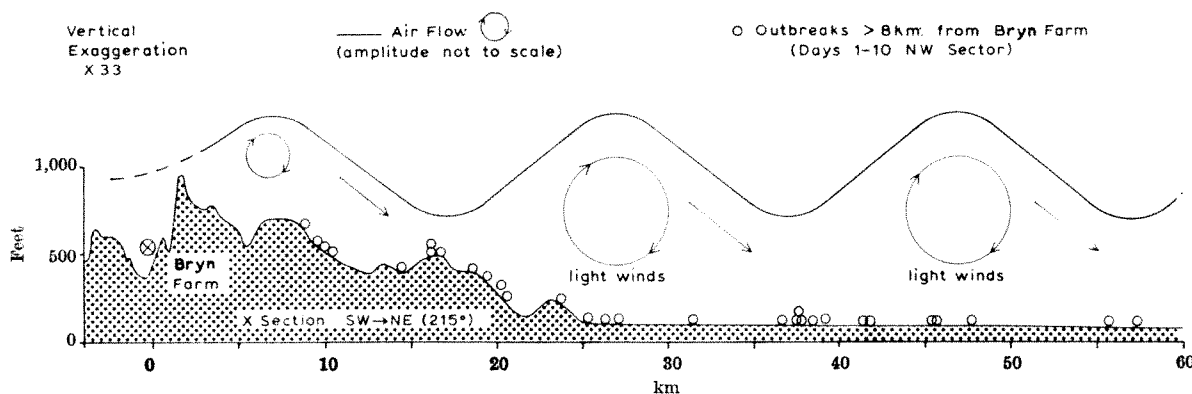


Fig. 2. Profile through Bryn farm illustrating the lee wave hypothesis.

the variable l^2 reaches a marked maximum at lower or middle altitudes and then declines rapidly with height. l^2 can be estimated from upper air soundings of temperature and wind speed. Soundings at Camborne (West Cornwall) were used in order to make sure the sample was that of the undisturbed flow over Bryn farm. Plots of temperature, wind speed and l^2 for 2330 h GMT on October 25 are given in Fig. 3. They show the classic symptoms of conditions favourable for the development of lee waves: an isothermal layer, wind speed increasing with height and a pronounced maximum in l^2 at middle altitudes⁹. Similar plots for 1130 h GMT on October 25 were also obtained. SIGMETs from the Flight Information Centre at Preston also predicted the occurrence of lee waves from 1630 h GMT on October 25 to 0430 h GMT on October 26 (personal communication from S. A. Casswell).

The wavelengths of the lee wave formations were calculated from mean tropospheric wind speed using Corby's regression equation which was derived from radiosonde data⁸ and has been confirmed by subsequent investigators^{10,11}. The list of wavelengths in Table 2 shows that lee waves on the afternoon and evening of October 25 would have had the required wavelength of 18–20 km.

Table 2. CALCULATED WAVELENGTHS FOR OCTOBER 25–26

Oct. 25 0530 h	11.1 km
1130	17.5
1730	20.4
2330	19.4
Oct. 26 0530	16.7
1130	11.7
1730	13.6
2330	9.5

Finally, it must be shown that the positions of the crests and troughs of any existing pattern with a wavelength of

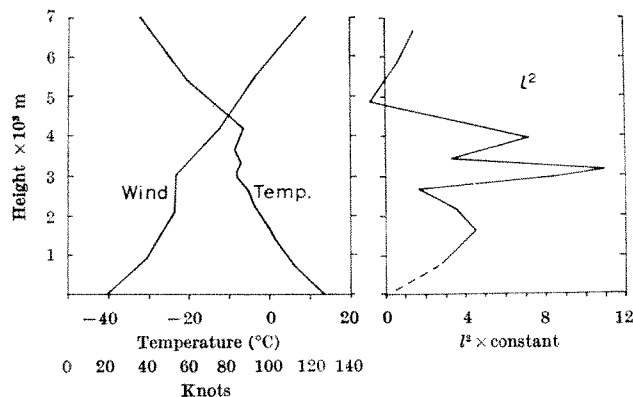


Fig. 3. Temperature, wind speed and l^2 for 2330 h on October 25, 1967, at Camborne.

about 20 km would conform to the positions shown in Fig. 2. The underlying topography can influence the amplitude and phase of the wave and so had to be taken into account in determining the position of the wave. Wallington's method¹² was used to calculate the streamline displacement, and the result is given by Fig. 2. Fig. 2 therefore represents atmospheric conditions as they probably were during the afternoon and evening of October 25.

Conditions at this time thus seem to have been conducive to the development of waves with a period of 18–20 km. The underlying topography would have ensured that the wave troughs in the air-stream formed over Bryn farm and over the downwind areas previously identified as "clusters". (The pattern of outbreaks in Fig. 2 was tested statistically with the method of Pearson¹³. The null hypothesis of randomness was rejected in favour of clustering at the 0.001 level.)

It seems that the lee wave hypothesis provides a plausible explanation of uptake of virus from a single (or localized) source, subsequent transmission at high concentration over long distances and deposition in relatively small areas downwind. Because neither primary outbreaks of foot and mouth disease nor lee waves are common events, the probability of a simultaneous occurrence of an initial outbreak at a site conducive to the development of lee waves and the occurrence of favourable conditions for lee waves seems quite low. It therefore seems that the probability of another similarly generated epizootic must also be quite low.

I thank M. E. Hugh-Jones of the Central Veterinary Laboratory, Weybridge; Dr R. Sellers of the Animal Virus Research Institute, Pirbright; Dr E. Stringer of the Department of Geography at the University of Birmingham, and various members of the Department of Geography at the University of Bristol for helpful comments on the draft of this article.

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Book Reviews

NEW LIGHT ON A LEGEND

The Birth of Penicillin and the Disarming of Microbes
By Ronald Hare. Pp. 236+8 plates. (Allen and Unwin: London, July 1970.) 63s.

THIS is a clearly written, non-specialist, autobiographical account of the medical discoveries and scientific research which the author has been associated with or has contributed to. His research career started with six years in Sir Almroth Wright's department at St Mary's Hospital. Then, at the new Queen Charlotte's Hospital, he and Colebrook successfully attacked puerperal fever by epidemiological means and routed it when 'Prontosil' and the sulphonamides became available. There followed several years at the Connaught Laboratories, Toronto, with work on influenza virus, aspects of preventive medicine, and, finally, the emergency large scale production of penicillin during the war. Thereafter, until his retirement in 1964, he was professor of bacteriology in the University of London, at St Thomas's Hospital. The intrinsic interest of the matter is more than sustained by the modesty and humour with which the book is written.

The five chapters dealing with Hare's period (1925-31) at the Inoculation Department, St Mary's Hospital, Paddington, are historically the most interesting; Hare was in close contact with Fleming throughout, and is therefore able to give an authoritative account of the circumstances surrounding his discovery of penicillin there in 1928.

Chance is often said to have played a key part in Fleming's discovery, but its role seems to have been even greater than is usually supposed. Thus it seems that Fleming had already discarded the famous contaminated plate, and it was only the chance arrival of D. M. Price before the tray of plates was removed which led Fleming to re-examine some of them and to make what turned out to be perhaps the most important casual observation ever made. The plates had been sown with staphylococci, and, on one of them, Fleming noticed that around a contaminating fungus (*Penicillium notatum*) the bacterial colonies were undergoing lysis. While this phenomenon of bacterial antagonism crops up occasionally, this particular pattern—of staphylococcal colonies undergoing lysis in the vicinity of a growth of *P. notatum*—can only be produced under very special conditions; thus contamination by the fungal spore must occur at or not too long after the inoculation with staphylococci, and the plate must not be incubated, but held for some days at a rather low room temperature. Again, it may have been fortunate that the occupant of the room below Fleming's, C. J. La Touche, should have been working with fungi. After his discovery Fleming tested the eight penicillia in La Touche's collection and found that one produced penicillin and that this resembled his own contaminant. Hare's theory that the latter came from La Touche's room by way of the draughty stairway is more satisfying than the legend of a spore "coming in through the window from Praed Street"—especially as the window was probably never opened.

To some, the most absorbing parts of the book will be the description of the conditions of work in the Inoculation Department, its atmosphere of purpose, dedication

and diligence, and the sidelights thrown on the legendary characters of Wright, Colebrook, Fleming and others. Perhaps it is here that partial answers may be found to the riddle of why Fleming did not pursue penicillin. To be sure, there were material reasons too; penicillin was unstable, tedious to prepare and purify, staphylococci could become resistant to it, and opinion generally was against the belief that antibacterial agents might exist which could be given parenterally. But the reason proposed by Maurois in his biography of Fleming, namely active discouragement by Wright, is strongly discounted by Hare. Another reason, given by Fleming, is that he was not a chemist. He had working with him, however, two young colleagues, F. Ridley and S. Craddock—the former trained as a biochemist. Their careful work, carried out under difficult conditions, showed that penicillin became more stable at a faintly acid pH, that it could be concentrated by vacuum distillation, and that a protein-free alcoholic extract of the concentrate contained most of the penicillin. This work was dismissed in three lines in Fleming's original paper, and was apparently forgotten; yet had Fleming done some simple *in vivo* tests with such material he would almost certainly have got results as striking as those obtained at Oxford eleven years later. In fact, the first material injected intravenously into mice in 1940 had been prepared in precisely this way, and even the big doses given had had little observable toxicity.

Perhaps one's astonishment that Fleming abandoned penicillin is partly founded on a fallacy, namely the often repeated legend—which Fleming himself did not correct—that he knew all the time that penicillin would turn out to have remarkable curative properties. According to Hare, after an initial period of enthusiasm, Fleming's hopes faded; his inaction only becomes understandable in this context. Nevertheless, the intriguing but unanswerable question remains—would somebody else in Fleming's position have persevered?

N. G. HEATLEY

PROBLEMS OF PLENTY

Seeds of Change

The Green Revolution and Development in the 1970s.
By Lester R. Brown. Pp. xv+205. (Pall Mall: London, April 1970. Published for the Overseas Development Council.) 60s.

THIS very readable book is concerned with the course and the consequences of the agricultural revolution that was initiated with the introduction into many developing countries of short, fertilizer-responsive and photoperiod-insensitive, varieties of wheat and rice. These varieties were initially produced by Rockefeller and Ford Foundation workers, in collaboration with local scientists, in Mexico and the Philippines respectively. The short rices give higher yields than their tall predecessors without lodging, and the same is true of the short wheats, provided that irrigation water and nitrogenous fertilizers are also available. Because of their insensitivity to photoperiod they can be sown at any time of the year in low-latitude regions and will develop normally to rapid maturity, so that multiple cropping becomes possible.

Wide publicity has been given to the sudden and massive increases in grain production that followed the transfer of the new varieties to Pakistan, India and other countries. The high yields depend always on the supply of water, to such an extent that yield per unit of water begins to make more sense than yield per unit of area. More than ever the capacity of the land to support people must be measured by its water supply rather than by its area.

But the benefits of the new varieties were often accompanied by difficulties created by inadequacy of storage and transport facilities, and sometimes by the social unrest produced by exaggerations of inequalities of income.

Greater demands for inputs of manufactured goods and services resulting from agricultural prosperity have created jobs outside the farm sector. This is helping to cope with the unemployment and underemployment that are unrelenting products of population growth. The overall economic growth that is the consequence of agricultural growth will also provide some of the necessary jobs. Mr Brown suggests that all increased production, whether agricultural or industrial, should be labour-intensive in order to provide the maximum employment. Unfortunately, it seems to me that this Gandhian concept is unlikely to provide a reasonable income for the workers because of their diminished individual productivity. Moreover, to keep the land in continuous production with multiple cropping each year, harvesting, land preparation and sowing must take the shortest possible time—so the balance between men and machines favours the latter.

If there are no shortfalls due to disease, pests or weather, Mexico, Kenya, Iran, the Philippines, Pakistan, Afghanistan and Turkey will have surpluses of their staple grains following the 1970 harvest. India, Malaysia, Ceylon and Indonesia should join them by 1973 on current projections. The surpluses will buy perhaps 15 more years in which to attempt to solve the people-food equation, according to Brown. This leads him to suggest that restraints on population growth are more likely to be achieved with the improved prosperity that would occur if markets could be found for the grain surpluses. Such markets would most likely be in the developed countries whose present levels of agricultural production are often sustained by governmental intervention in the form of price support or tariff protection. Brown would like to see these policies discontinued. This argument will be hard to accept in the developed countries because the surpluses of the potential exporters are of uncertain extent and reliability, and population growth may well limit the period that the surpluses persist, while the quality of the new wheats and rices will restrict their acceptance on world markets. On this basis it would be unwise speedily to dismantle systems of agricultural policy and production that could be reconstructed only with great difficulty and considerable delay. At the same time, those who provide the secondary products and services for developing countries with grain surpluses will clearly wish to assist in the disposal of the surpluses on world markets and to consider the sources of their own imports.

Seeds of Change is a book of hope and of warning. It shows what the application of crop technology is doing to the developing world: telling of the initial relief and warning of the further actions that are necessary if we are not once more to regress into famine. It will be read with interest by agricultural research workers, by agriculturalists, economists and politicians.

RALPH RILEY

RECORDS OF POLLUTION

Environmental Pollution

Edited by Kenneth Mellanby. Vol. 1, No. 1. Quarterly. (Elsevier: Barking, Essex, July 1970.) 130s per annum.

Just a few years ago, the only journals with a declared interest in pollution were either medical or purely technical. There is now a flood of periodicals concerned with the state of our environment, some clearly propagandist or frankly cranky, while many of the more sober publications cover rather a wide range. Of these, *Biological Conservation* has, in the past, accepted a number of papers dealing with the ecological effects of pollution. *Environmental Pollution* was launched by the same publishers to specialize in such contributions from social, economic and engineering viewpoints as well as the biological, leaving its sister journal to deal with the management of

biological resources and the remedying of pollution damage. The high standard of the journal, were it in any doubt, is made plain by the list of its editors; Dr Kenneth Mellanby heads a board which includes Professor R. W. Edwards of Cardiff, Dr Gordon Goodman of Swansea and Dr J. E. Smith of Plymouth among its British members, together with ten eminent overseas scientists.

The first of four quarterly numbers contains four original papers and one review within 77 text pages, followed by two fairly lengthy book reviews. One paper is American and the remainder British; the journal will no doubt acquire a more international flavour as it becomes better known. Contributions will be accepted in French and German, when English summaries are required. Overseas readers may be annoyed to find that the English papers in this number, by contrast, do not include summaries in these languages. There are not only the standard "Instructions to Authors" inside the back cover but also "Notes for Authors" at the end of the text pages; the two are not entirely consistent. The more detailed "Notes" demand that all illustrations be submitted in duplicate, which seems not only a considerable burden in the case of an elaborate diagram but a quite unnecessary one. Perhaps a reduced copy is meant, such as most experienced authors submit for the use of editors or referees. The other instructions regarding illustrations are similarly confusing rather than helpful. I spend some time advising students on the preparation of their illustrations and should like to issue a general plea to journal publishers on their behalf to make these instructions not only clear but also reasonable.

The first paper is by a trio of workers at the editor's research station, Monks Wood, and deals with the presence and toxicity of polychlorinated biphenyl compounds in British wild birds—a very topical subject, in view of the partial responsibility assigned to PCBs for heavy bird mortalities in the Irish Sea last year. Jenny Baker's review of the effects of oils on plants is one result of her work at the Orielton Oil Pollution Research Unit and refers to eighty-nine sources, some of which are themselves shown as having large bibliographies; it is thus a very useful piece of background literature. F. B. Pyatt follows up the surveys of Fenton and later workers elsewhere with a study of the effects of air pollution on the distribution of lichens in the steelmaking town of Port Talbot. Further work from Monks Wood, the effect of DDT on tadpoles, is reported by A. S. Cooke and is followed by a paper from Professor Feder of Waltham, Massachusetts, on plant response to chronic ozone pollution.

A very subjective indication of the value of *Environmental Pollution* is that the review and two of the papers are of direct interest to me, while another of the papers may be indirectly useful. There is every sign that the value of this journal to workers in the field of pollution will increase, and it is to be hoped that its readers will include technologists as well as natural scientists.

A. NELSON-SMITH

HAZARDS FROM SMOKING

Inhalation Carcinogenesis

Edited by M. G. Hanna jun., P. Nettesheim and J. R. Gilbert. (Proceedings of a Biology Division, Oak Ridge National Laboratory Conference held in Gatlinburg, Tennessee, October 8–11, 1969.) (AEC Symposium Series, No. 18.) Pp. xii+524. (Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, US Dept. of Commerce: Springfield, Virginia, 1970.) \$3.00.

Of all human organs, the lung offers the greatest surface area to the environment, and among British males the lungs are the most common sites of cancer. Inhalation carcinogenesis, the subject of this US Atomic Energy Commission symposium, is therefore of great importance

from the point of view of human health. Although most of the contributors are American the published papers give a reasonable account of the subject as it stood about a year ago. The hazards of inhalation carcinogenesis arise chiefly from occupational exposure, air pollution and smoking. It seems, however, to be more feasible to reduce the exposure from occupations and air pollution than that from smoking.

Until recently the association of exposure to inhaled toxic materials and the development of lung cancer was based solely on epidemiological and statistical findings. The papers given at the symposium are chiefly concerned with experimental carcinogenesis by gases, dusts and particulate clouds. The study of the biological effects of inhalation involves more sophisticated physical methods than are involved in the use of other routes of administration.

In the opening paper Professor Norton Nelson discusses the possible causes of lung cancer in man including radioactive materials, chromates, mustard gas, nickel, arsenic, asbestos and cigarette smoke. Miners' disease which Joachimsthal and Schneeberg identified as lung cancer in 1879 was one of the earliest recognized environmental cancers: lung cancer is still an occupational hazard among the uranium miners of Colorado. The first experimental lung cancers were induced with radioactive materials (^{144}Ce) by Lisco and Finkel and these and later experiments with radionucleotides are described by Dr W. J. Bair.

Some of the communications describe the techniques suitable for inhalation studies, the equipment for producing defined and controlled aerosols and mixtures of gases, particularly oxides of nitrogen. The design and construction of inhalation chambers and of cigarette smoking machines are described by several authors. Other papers describe experiments in which radioactive or chemically carcinogenic materials were implanted in different parts of the respiratory tract; although lung tumours have been induced by these techniques, they are not really caused by inhalation.

The effects of inhaled material include biochemical changes, such as denaturation of collagen fibres by ozone or nitrogen dioxide described by Buell, and an increase in DNA synthesis in the lungs of hamsters exposed to cigarette smoke found by Boren. Such exposure also causes lysis of lysosomes. The irritant gases, nitrogen dioxide and ozone, reduce resistance to infection by *Klebsiella pneumoniae* (Ehrlich, Henry and Fenters) or *Streptococcus C* (Coffin).

Laskin, Kuschner and Drew have induced carcinomas in rats by exposure to clouds of carcinogenic benzopyrene and irritant sulphur dioxide, but not by exposure to either carcinogen or irritant alone. Montesano, Saffiotti and Shubik induced bronchogenic carcinomas in hamsters by intratracheal instillation of benzopyrene and finely dispersed ferric oxide, but not with either chemical alone. These studies suggest that the combined action of irritant and carcinogen is necessary to induce lung tumours in animals. Auerbach, Hammond, Kirman and Garfinkel allowed dogs to smoke cigarettes through a tracheostomy tube. After the first attempts, the dogs seemed to enjoy smoking and smoked up to twelve cigarettes per day. Animals killed after 420 days had pulmonary fibrosis and emphysema and reports later than those under review have indicated that the procedure has induced bronchogenic cancer in some of the dogs. These are the first successful experiments to resemble the human experience with cigarettes.

The papers show that advances are being made in inhalation carcinogenesis and the experimental models for the genesis of lung cancer in man should make it possible to reduce the incidence of the disease by defining the environmental hazards and perhaps making it possible to manufacture less dangerous cigarettes.

E. BOYLAND

METABOLIC DISORDER

Early Diabetes

Edited by Rafael A. Camerini-Dávalos and Harold S. Cole. Associated Editor W. S. Gailmor. (Advances in Metabolic Disorders, Supplement 1.) Pp. xxi+486. (Academic: New York and London, March 1970.) 215s.

THIS book reports a group exchange of views of the First International Symposium on Early Diabetes. It is a monument to the fact stated in the first chapter of the book that "despite an enormous amount of work, there is still much confusion and disagreement concerning the primary lesion or lesions in diabetes mellitus". The galaxy of cross currents and statements, which are often contradictory in this investigation of diabetes, reflect the true state of the art. For those interested in becoming involved in these turbulent waters, the facets covered in the chapters dealing with genetics of diabetes in relation to animal and man, the function and control of the secretions of islet cells of the pancreas in normal and abnormal animals, and studies on insulin secretion in man would be valuable. The maternal-placental-foetal relationship in pregnancy and the association with carbohydrate intolerance is investigated, followed by definitions of pre-diabetes and latent or chemical diabetes. It is sad to relate that the natural history of diabetes occupies only 20 pages compared with 78 pages dealing with the treatment of early diabetes. This probably reflects the attitudes of those organizing the meeting rather than the real state.

This is not a book for the uninitiated or the tyro, but gives the new research worker an idea of the currents of interest and conclusions of past work. It is essentially a historical piece, full of the imperfections and changes that are inevitable in a subject which is rapidly changing. It gives little guide to the clinician engaged in the patient care field of better or improved approaches to treatment of this disorder. Fundamental to any research, however, is a knowledge of the approaches in the past that have not worked. This is an essential requirement for the research worker in metabolism and this book is recommended to them.

JOHN ANDERSON

POPULATION GENETICS

Evolution and the Genetics of Populations

Vol. 2: The Theory of Gene Frequencies. By Sewall Wright. Pp. vii+511. (University of Chicago: Chicago and London, April 1970.) 135s.

POPULATION genetics, like any other branch of science, is growing exponentially. It would be hoping for too much that all of the mathematical theory could be compressed into a book even of this size; for example, some of the important work of Kimura, Ewens, Malécot and others is referred to rather briefly. But, bearing this almost inescapable limitation in mind, it is truly astonishing how much is to be found here. The theory of gene frequencies began early this century with the observations of Yule, Castle, Hardy and Weinberg. If genes G, g have respective frequencies p, q , then in a large random mating population the genotypes GG, Gg, gg occur in the ratios $p^2 : 2pq : q^2$. This simple formula will in practice be modified by the occurrence of multiple alleles G_1, G_2, \dots, G_n , at one locus, by linkage of interaction between different loci, sex linkage, by mutation, selection, differential fertility, migration, inbreeding and assortative mating, cytoplasmic factors, and random fluctuation from generation to generation. Put together, these lead to an immense variety of possible situations.

Useful questions to ask are how a population will change under these combined influences, whether it will stay close to a "stable equilibrium" for long periods, how

far the composition of the population is liable to fluctuate under random change, and what are the correlations between relatives and the components of variance. These topics are dealt with in great detail, bringing the reader largely up to date in developments in the field and indicating where further information can be found. This is no small achievement in itself; what is more, a very considerable part of the contents is Sewell Wright's own work, spanning more than 50 years. He invented the methods of path coefficients, the inbreeding coefficient F , and the surface of adaptive values of mean fitnesses whose peaks indicate (nearly enough) stable states of the population. These techniques run through the book as unifying threads, though other methods of tackling the problems (such as the matrix theory of Fisher and Haldane) are also mentioned. The book gathers together information spread throughout an ever-increasing mass of literature, so that it will be a most useful reference book for all whose work concerns population genetics.

CEDRIC A. B. SMITH

BIOCHEMICAL GENETICS

The Principles of Human Biochemical Genetics

By Harry Harris. Pp. ix + 328. (North-Holland: London and Amsterdam, 1970.) 117s (\$14.00) boards; 53s (\$6.30) paper.

Books by Professor Harry Harris have a creative aspect altogether their own. In 1953 he published *An Introduction to Human Biochemical Genetics* and this was almost certainly the first time the term human biochemical genetics was used. It was a milestone in the development of that subject; nothing like it had appeared since Garrod's last book in 1923. In 1959 followed Harris's textbook *Human Biochemical Genetics* which firmly established the new discipline. At that time it was possible to cover the field completely, but, in 1970, one book can no longer do this and the new work has become *The Principles of Human Biochemical Genetics*. Nevertheless, these principles are illustrated by examples, many of which are taken from human haemoglobins and certain enzymes such as glucose-6-phosphate dehydrogenase and serum cholinesterase, and at one place or another almost all aspects of human biochemical genetics are referred to. For example, there is a table listing thirty enzyme and protein polymorphisms, supported in each case by the methods of detection and references. Another table lists twenty-five disorders caused by specific enzyme deficiencies (inborn errors of metabolism); any enzyme deficiency which has not been discussed elsewhere is briefly annotated and again references are given.

There are chapters on gene mutations and single amino-acid substitutions, the "one gene one polypeptide chain" theory and its implication, on duplications and deletions and their effects on protein structure, on gene mutations affecting the rates of protein synthesis, and on the quantitative and qualitative variations of enzymes. Other sections of the book are more specific and deal with inborn errors of metabolism, the enzyme and protein diversity in human populations, the blood group substances, the molecular pathology of inherited disease, the effects of dominance and recessivity, heredity and environment. The illustrations are ingeniously simple, and together with the tables, often throw an entirely new light on a problem. This applies, for example, to the felicitous way Harris lists the haemoglobin variants, how he illustrates the production of hybrid proteins, and how intragenic crossing-over may give rise to haemoglobin Lepore in the case of haemoglobin, or to the appearance of a triplicated allele in haptoglobins.

Harris states in his preface that this book has grown out of a course of lectures given at the Galton Laboratory,

intended not only for those specifically studying human genetics but also for biochemists, biologists and medical men. It is exactly that circle of readers which will find not only information but also inspiration in this book.

It is worth mentioning that the book is excellently produced and the paperback is good value at 53s.

H. LEHMANN

VISUAL PERCEPTION

Perception Through Experience

By M. D. Vernon. (Methuen's Manuals of Modern Psychology.) Pp. x + 306. (Methuen: London, May 1970.) 55s.

THE title of Professor Vernon's third book on visual perception catches the attention. When we read in the introduction that "knowledge and experience are particularly involved in the inferences we commonly make as to the nature of objects and events", we look forward to reading about how experience does enable us to make these inferences about perceived objects. This sort of issue is now widely recognized as being central to the problem of perception. How is it that from the fragmentary and disjointed data arising from fleeting two-dimensional patterns on the retina, we have the overwhelming impression of a stable world filled with people and things? Clearly, systems of perception interpret sense data as objects. Equally clearly this process requires a contribution from within, of stored knowledge about the nature of the world, which must be many times greater than the contribution made by sense data at any one moment. It is the interpretation of the sense data in terms of our knowledge which we see, not the sense data themselves.

Professor Vernon does not set out to explain how these processes occur. Instead she reviews a fairly wide range of experimental literature, doing "little more than allude to the various theories which have been advanced". She does cover a lot of experiments, however, ranging from the recent work of Fantz and Bower on the visual abilities of very young children, through attentional processes and some of the standard perceptual topics such as the constancies and seeing movement, to the relation of motivational and personality characteristics to perception. Rather surprisingly, in view of the title, there is not a great deal about perceptual learning, although there is quite a lot of material on children.

The book is said to be intended for students taking degree courses in psychology. Though it contains descriptions of many interesting experiments, I rather doubt whether undergraduates have the experience to perceive the interest or importance in experimental studies until the data are embedded in reasonably well articulated explanations and theories. The author takes a different view, arguing that extensive knowledge, which she here attempts to provide, should precede theoretical argument.

KEITH OATLEY

FAT SOLUBLE VITAMINS

Fat Soluble Vitamins

Edited by R. A. Morton. (International Encyclopaedia of Food and Nutrition, Vol. 9.) Pp. xii + 530. (Pergamon: London, Oxford and New York, April 1970.) 120s; \$16.

THIS is the second to appear of the twenty volumes of the "International Encyclopaedia of Food and Nutrition" now in preparation. Volumes are being issued as they are completed and without reference to their position in the series.

Professor R. A. Morton has assembled a distinguished team of authors to review all aspects of the chemistry and biochemistry of the fat soluble vitamins and their significance, role and use in human and animal nutrition. After an introductory chapter setting the field in perspective, chapters are devoted to the chemical structure and physical properties of the vitamins, their biosynthesis and their bioassay. The biochemistry of vitamins A, D and K is discussed in separate articles and there is a comprehensive chapter on the tocopherols. The mode of action of the fat soluble vitamins is still largely unknown, but the well established role of vitamin A in vision is discussed in some detail. By way of digression an article is given to lipoic acid which, although not generally classed as a vitamin because it is not required by higher animals, is a growth factor for some microorganisms and has a clearly defined biochemical role. The remaining chapters are concerned with the more technological aspects of the synthesis and utilization of the fat soluble vitamins and with their application in practical nutrition.

This volume is a useful and self-contained book. The individual chapters are well written and, although differing somewhat in their breadth and depth of treatment, they all include adequate references to sources of further information. Unfortunately it is evident that most of the articles were completed during 1967. While it is recognized that a book of this sort can never be absolutely up to date, it is clear that the long term value of the encyclopaedia will largely depend on the ability of the editors to carry out their intention to provide continuous up-dating by producing an annual review to report recent advances in the fields covered.

J. W. G. PORTER

PHILOSOPHY AND ATOMIC PHYSICS

Atomic Order

An Introduction to the Philosophy of Microphysics. By Enrico Cantore. Pp. xi+334. (MIT: Cambridge, Massachusetts, and London, April 1970.) 117s.

THE aim of the book is to contribute towards a better understanding between the humanist and scientific cultures, and the specific problem discussed is the lack of communication between philosophers and scientists. The method adopted is called inductive-genetic; that is, the investigation is limited to a well established scientific discipline, namely atomic physics, and the intention is not only to examine the consistency and completeness of quantum theory, but also to show how the gradual gathering of experimental evidence has necessitated the formation of the theory. The investigation is meant to serve as the basis for discussing the interdependence of science and philosophy.

In the first half of the book, the author gives a survey of atomic physics, and, first of all, he succeeds in explaining how quantum theory is able to account for all the evidence about atoms and atomic aggregates. Calculations are omitted, but essential experimental results are amply presented in tables and diagrams, and there are plenty of references for further readings. As to the philosophical analysis, it is essentially ontological. According to the author, to observe an atomic object it is necessary to disturb it; that is, the object must interact with the instruments of observation, and, because of the existence of the fundamental quantum of action, we have uncertainty relations. But what is then the atomic object, the undisturbed state of which it is fundamentally impossible to observe? To the author it seems to be an essentially classical particle, for, when talking about the wave function representing the atomic object in quantum theory, he states that the microentity certainly does not fill up the entire volume occupied by the wave, but remains a concentrated piece of matter all the time (p. 241). In fact, this idea is further developed using the concept of local motion,

which is considered to be just as real at the atomic level as in classical mechanics. But the author totally fails to convince the reader that this conception of the atomic object is concordant with the experimental basis of wave mechanics; that is, with the basis of quantum theory itself.

The principle of complementarity is very sketchily discussed, but it is said to be very important to the philosophical considerations. It seems, however, as if the author has not grasped the principle fully, because he persists in speaking of disturbance of atomic objects by observation. According to Bohr, this way of talking should be avoided because it implies the existence of a world of undisturbed physical objects, which is fundamentally non-observable. An analysis of Bohr's phenomenon-centred terminology would have been appropriate, at least as an alternative to the ontological point of view.

Thus I find the philosophical part of the book rather confusing and also tedious. The same ontological viewpoints are repeated under different headings, but without clarification of the central epistemological problems.

T. BERGSTEIN

ACCELERATOR PHYSICS

Intermediate Energy Nuclear Physics

By W. O. Lock and D. F. Measday. Pp. xiii+320. (Methuen: London, April 1970.) 85s.

THIS book is a revised and expanded edition of *High Energy Nuclear Physics* by W. O. Lock. It deals chiefly with the nuclear and elementary particle physics which can be explored with accelerators in the energy range 100–1,000 MeV. The change in title does not reflect a change in content so much as the change in accelerator technology which has occurred during the past 10 years. The book is intended as an introductory text for postgraduate students.

The first four chapters contain a mixture of basic nuclear and elementary particle theory, a section on accelerators and experimental techniques and one on the properties of the electrons, muons, neutrinos, pions, nucleons and some other light mesons and baryons. In the next four chapters the experimental evidence is given on the interactions of pions, photons, electrons and nucleons with nucleons. The last three chapters describe the interactions of photons, electrons, muons, pions and nucleons with nuclei.

This is an extremely wide field to cover in a single book and there is not enough space to give more than a superficial treatment of many of the topics. It therefore seems a pity that some space has been used to reproduce basic theory which can be found in most books on quantum mechanics. The most useful chapters will probably be those on the interactions between elementary particles and nucleons, and here the text is supplemented by many graphs of experimental results. The experiments are discussed in the light of predictions of modern conservation laws and symmetries. A useful feature is the bibliography and the long list of references given for each chapter.

It would have been more satisfactory if certain sections of this book had either been expanded considerably or left out, but many people will find it useful as a rather broad review of the subject.

R. C. BARRETT

FORMULAE FOR ASTROPHYSICISTS

Elementary Processes for Cosmic Ray Astrophysics

By V. L. Ginzburg. (Topics in Astrophysics and Space Physics, No. 1.) Pp. viii+131. (Gordon and Breach: New York and London, April 1970.) 105s (\$12.60) boards; 70s (\$8.40) paper.

THIS book is based on the lecture course which the author prepared for the 1966 Summer School in Les Houches, but which was never actually presented, with the addition

of a separate section giving a comprehensive and more up to date treatment of synchrotron emission and reabsorption. The rest of the book covers radiative processes—the Compton effect, thermal bremsstrahlung radiation, ionization losses and collective plasma processes—thoroughly, but in rather less detail. Particle production receives only a very brief treatment, and the discussion of the generation and absorption of γ and X-rays receives less prominence than one might expect from the title of the volume.

On the whole, this is a useful book which gathers together many of the formulae required by those working in the fields of high energy astrophysics. But once again the relatively high cost of what is a rather slim volume requires comment. The price is not excessive by the standards of Gordon and Breach, but it is difficult to see why these standards should differ so much from those of other publishers, particularly when the physical construction of the book and the incidence of minor misprints suggest that no correspondingly high standards were maintained in its manufacture.

JOHN GRIBBIN

GEOMORPHOLOGY OF THE SAHARA

L'Évolution Continentale Post-Hercynienne du Sahara Algérien

(Saoura, Erg Chech Tanezrouft, Ahnet-Mouydir.) By Georges Conrad. (Centre de Recherches sur les Zones Arides: Série Géologie, No. 10.) Pp. 527+11 plates. (Editions du Centre National de la Recherche Scientifique: Paris, 1969.) 120.40 francs.

THIS well organized and handsomely illustrated *thèse* deals with the geomorphological evolution of a large segment of the north-western Sahara, between the Atlas of Western Algeria and the north-western slopes of the Hoggar.

After dealing with the geological antecedents (Palaeozoic to early Tertiary), the core of the study considers successively the late Tertiary, the Plio-Pleistocene, the early, middle and late Pleistocene, and the Holocene. A series of excellent sections is here used to describe patterns of sedimentation or erosion, with a series of informative palaeogeographic reconstructions. During this period of time, climate fluctuated within a range of hyperarid, arid, subarid and semiarid, with an overall trend to greater aridity and less accentuated oscillations. Different sedimentary and pedogenetic processes are repeatedly identified for the northern and southern parts of the study area, presumably reflecting latitudinal contrasts of climatic change. So, for example, during the course of the Pleistocene, ferricretion among the Hoggar foothills repeatedly corresponded to calcerection in the Saharan lowlands and on the Atlas slopes, while two phases of silicification during the later Tertiary were restricted to the southern half of the region. Part two serves as an appendix to describe particular sediments and soils: torba (fine Tertiary basin fills consisting of attapulgitic, carbonates and detrital quartz); evaporites; lacustrine dolomites; calcareous and ferruginous crusts; silcretes; daia (sink-hole depressions) fills; eolian sands; fech-fech (powdery, fine but heterogeneous surface deposits rich in soluble salts and lime dust); and clay minerals. The final section provides a general synthesis, with comparative stratigraphy from other parts of the western Sahara, the Chad Basin and the Senegal Delta (including a detailed chart and list of ^{14}C dates).

This volume is very welcome, providing a unique documentation of the late Cainozoic of the north-western Sahara. Even if matters of stratigraphy or interpretation require revision in the future, the wealth of objectively presented data will remain valuable to all interested in the Saharan world.

KARL W. BUTZER

Short Notices

Women in the Field: Anthropological Experiences. Edited by Peggy Golde. Pp. 343. (Aldine: Chicago, July 1970.) \$8.95.

THIS fascinating anthology of experiences, opinions and theories by thirteen eminent lady anthropologists was conceived as a guide for students who are pondering how and where they should pursue their studies of man and his ways. Each contribution is a personal account of one woman's experiences as an outsider breaking into a strange culture. The restriction to one sex is no empty gimmick—women face special problems in alien societies, and, in any case, it is worth considering their place in a profession that seems to have done well for a relatively large proportion of them. Clearly it is a profession in which rewards are hard won; there are hair raising dangers and depths of despair in plenty in this book. Ruth Landes was accused of being both a prostitute and a communist spy in Brazil, and Peggy Golde retreated to the church as the only place in a Mexican village where she could have a cry. Margaret Mead, however, reports no such hostility in New Guinea. As an insight into the motivation and way of life of the woman anthropologist in action, this book is excellent, far better than its sober cover and rustic title suggest.

Gilbert White's Journals. Edited by W. Johnson. Pp. xlviii+463. (David and Charles Reprints: Newton Abbot, May 1970. First published in 1931.) 105s.

WITH the modern fashion for centenary celebrations and the like of one sort and another, the publishers of reprints have an assured market and it can be no coincidence that a reprint of Walter Johnson's selection from Gilbert White's *Naturalist's Journal* is published this year, the 250th anniversary of White's birth. For devotees of the *Natural History and Antiquities of Selborne*, who missed Johnson's 1930 edition and who cannot manage a visit to the British Museum to see the original manuscripts, here then is a chance to buy a partner for White's classic. They will not be disappointed—the *Journal* was used by White as a sourcebook for his *Natural History*, and it abounds with similar delightful and acute observations of life around him. While commenting daily about the birds and the bees, Timothy the tortoise, his neighbours and other goings-on in the nearby gardens and fields, White also slips in the unexpected, as on May 4, 1778, when he complains about the five running firings on the day the King and Queen visited the fleet at Spithead, which "shook my house and made the windows jar": Noise Abatement Society take note.

Permafrost in Canada: Its Influence on Northern Development. By R. J. E. Brown. (Canadian Building Series, 4.) Pp. viii+234. (University of Toronto: Toronto; Oxford University: London, July 1970.) 120s.

IT is a chilly thought that half of Canada's land surface lies in the region of permafrost or perennially frozen ground—ground which for several continuous years remains below freezing point. The layer may vary in thickness from only a few inches to more than 1,000 feet in the extremes, the thickest recording in Canada being 1,500 feet at Winter Harbour on Melville Island in North West Territory. Until recently, such inhospitable land hampered all but the slightest development, but, as is shown in this book, it is now technically possible to build almost any engineering structure in the Canadian permafrost—roads, railways, bridges, buildings, and the like—even in the worst regions, though its construction cost is considerably greater there than in more temperate climes. In a comprehensive survey of the nature of permafrost and of its effect on the development of Canada's frozen north, Dr Brown shows that, in his search for greater wealth, man's activities have no bounds.

Correspondence

Geophysical Theory

SIR,—May I, while thanking Professor Runcorn for the greater part of his review of the new edition of *The Earth* (*Nature*, 227, 525; 1970), reply to his critical remarks concerning certain parts of it?

He refers to my "robust prejudices" and states that I indulge in "selection (selective?) quotation". I admit to a prejudice, namely that in an alleged explanation the conclusions should follow from the hypotheses, and that if the hypotheses lead to conclusions different from the facts there is something wrong with them. This prejudice is shared by most scientists.

As for selection, I admit that I have not read everything published in support of continental drift. I think that I have given reasons why the alleged explanation does not explain things that have happened and explains too many things that have not happened. On the other hand, I have not seen any work by a supporter of drift that even mentions that there are difficulties.

From Runcorn's review it would be inferred that I have not treated imperfection of elasticity apart from fracture. Following on the work described in the book (p. 331 *et seq.*) Crampin and I^{1,2} have recently published further work, and the form that we find forbids convection and continental drift. It gives quantitative explanations of facts far beyond the original data. Most seismologists concerned with damping use a law that departs even more than ours from one type that permits convection.

Since the final proofs were passed, there have been extensive and severe criticisms of continental drift from the geological point of view by Meyerhoff³ and Biswas⁴.

Yours faithfully,

HAROLD JEFFREYS

St John's College,
Cambridge.

¹ Jeffreys, H., and Crampin, S., *Mon. Not. Roy. Astron. Soc.*, **147**, 295 (1970).

² Jeffreys, H., *Nature*, **225**, 1007 (1970).

³ Meyerhoff, A. A., *J. Geol.*, **78**, 1 (1970).

⁴ Biswas, B., *Rising Continents, Deepening Oceanic Basins, and their Changing Configuration* (B. Biswas, Calcutta, 1970).

Wayward Bacterium

SIR,—Permit me to call to your attention evidence of a misconception on the part of your writer responsible for the article "Lunar Bacteriology—Bacillus by Rocket" (*Nature*, 226, 1000; 1970). The question is asked, "But how did the bacterium escape through the tight sterility net applied to all extraterrestrial space missions?"

Early in the decade of the 1960s there was concern for terrestrial contamination of the Moon; but as the matter was studied, it became apparent that, though terrestrial life might survive on the Moon, it could not multiply in that adverse environment, and it could therefore be no threat to lunar life if it existed. Subsequently the only biological constraint on lunar missions has been the expressed opinion of the International Committee on Space Research (COSPAR) that careful sterilization is desirable for drills designed for deep lunar subsurface boring. NASA has gone one step further, however, and on the basis of a recommendation by the Space Science Board of the National Academy of Sciences has kept its lunar landing hardware as biologically clean as was practi-

cal. This action resulted in approximately 5×10^6 viable spores being aboard Surveyor 3 at the time of launch, as compared with 1×10^8 to 1×10^9 spores for a spacecraft assembled without cleanliness controls.

In contrast is the constraint placed on planetary missions. COSPAR has recommended that launching states assure that there shall be only one chance in one thousand of contaminating a planet deemed important for the investigation of extraterrestrial life during the period of biological exploration. NASA has closely adhered to this requirement by biasing the trajectory of non-sterile flyby missions away from the planets sufficiently so that the probability of direct impact is very small, and no ejecta can reach the atmosphere. When missions are launched to land capsules on Mars, those parts of the missions intended to land on the planet's surface will be sterilized to the extent that they will have a probability of less than 1×10^{-3} of contaminating the planet.

The United States is very aware of and is actively engaged in meeting its responsibility to protect the planets from biological contamination carried on its spacecraft. Its responsibilities with regard to the Moon have been more than met.

Yours faithfully,

LAWRENCE B. HALL

Planetary Quarantine Officer,
Bioscience Programs,
Office of Space Science and Applications,
National Aeronautics and Space Administration,
Washington DC 20546.

French Nuclear Tests

SIR,—The welfare of South Pacific communities is increasingly at risk in the face of relentless testing of nuclear weapons in French Polynesia. Public outcry in Europe prevented France from continuing her test programme in Algeria. However, the protesting voices of small South Pacific governments have been ignored.

We are faced with increasing evidence of the hazards of uncontrolled radioactivity. While the extrapolations of Sternglass¹ may overstate the threat, we cannot be complacent. Similar, more moderate, warnings by Gofman and Tamplin² and others on the dangers of the infamous "permissible" level of radiation are generally accepted in the scientific community.

While the lasting danger to the people of the South Pacific lies in a general atmospheric contamination which will be shared, to an extent, with the rest of the world, a more immediate threat exists from contaminated fish. Certain of the large migratory fish such as tuna might feed on smaller fish dependent on heavily contaminated plankton drifting from the test area. Such deadly migratory fish could turn up in catches all over the Pacific.

Coral reef organisms have a great capacity for concentrating radionuclides. Data of Odum and Odum³ show a thousand-fold concentration in coral. Molluscs also are notoriously efficient concentrators of radionuclides and these organisms constitute a major part of the diet of South Pacific Islanders.

The French Defence Minister, M Debré, while in Tahiti recently, had the audacity to say that atomic scientists had "proved that the nuclear tests left no radioactive contamination in the area". British, New Zealand and

Australian government reports⁴⁻⁶ give extensive data to the contrary and R. S. Cambray (personal communication), senior author of the British report, points out that about 75 per cent of the long-lived activity in the southern hemisphere in 1969 was due to the 1968 nuclear tests. This percentage is presently rising as the 1970 series nears completion.

Can the scientific community remain complacent in the knowledge that the French government intends to continue testing in the South Pacific next year, and under conditions of reduced safety? M. Debré has underlined his government's lack of respect for the people of the area by recently announcing a reduction of what he terms "the surfeit of useless precautions".

I call on responsible scientists to use their influence, through the various scientific organizations, to enlighten their governments on the radiation hazards to which South Pacific communities are exposed and to impress on these governments the need to pressure France, as a humanitarian gesture, to cease nuclear testing in the South Pacific.

Yours faithfully,

GRAHAM B. K. BAINES

School of Natural Resources,
University of the South Pacific,
PO Box 1168, Suva, Fiji.

¹ Sternglass, E. J., *New Scientist* (July 24, 1969).

² Gofman, J. W., and Tamplin, A. R., *Environment*, **12**, (3), 12 (1970).

³ Odum, H. T., and Odum, E. P., *Ecol. Monogr.*, **25** (3), 291 (1955).

⁴ Cambray, R. S., *et al.*, Report, *AERE R6212* (HMSO, 1969).

⁵ National Radiation Laboratory, *NZ Dept. of Health Report No. NRL-F33*, (1969).

⁶ Gibbs, W. J., *et al.*, *Austral. J. Sci.*, **32**, 238 (1969).

Journal Dissemination

SIR,—One major impediment to the further development of science and advanced technology in developing and underdeveloped nations is that scientific journals reach us much more slowly than they reach laboratories in the developed nations. There are two reasons for this: our library budgets are small, so that to maintain a maximum coverage we are limited to seamail subscriptions. Second, in many cases the postal services are so bad that even after the journals reach the country they may be delayed several months before reaching the laboratory.

It has been suggested that the IATA might accept the shipment of scientific journals at reduced rates, but this piece of special pleading would, we suppose, be resisted since many other people may also feel that they merit special consideration.

We make the following proposal: that every scientific journal should be published in two forms simultaneously, in the present form and in microfiche (or microfilm). Any subscription to the journal would automatically consist in a subscription to one copy in each form. The microfiches could easily be sent by airmail letter post (this would be much faster even than present airmail subscriptions because letters receive very much higher

priority than parcels or printed matter even when the latter are sent airmail) at a very low cost and if the conventionally bound journal took several months to arrive by sea it would not matter so much. The additional cost of making the microfiches centrally and in such numbers would be very small and the problem of copyright would not be more acute than at present since the microfiches would be sent only to people who receive the journal by subscription in the normal way.

We are aware that certain journals are already offered in both forms but with the subscriber having to make the choice between them. We believe that our proposal is superior and that the additional cost would be sufficiently small that the price of subscriptions need not be raised significantly.

Finally, we feel that the proposal would also benefit science libraries with more favourable postal services since they would need to keep the bound journals only for a year or two while keeping a complete record of the literature on microfiche.

Yours faithfully,

ENRIQUE GRÜNBAUM
CLAUDIO GONZÁLEZ

Sociedad Chilena de Física
(Socifi), Casilla 653,
Santiago, Chile.

Citation Indexing for Studying Science

SIR,—E. Garfield's list (*Nature*, **227**, 669; 1970) of authors most cited in 1967 features a selection of authors of techniques for cytology, which prompts comment on the objectivity he claims for citation indexing, in the light of current writing and editing practice in this field leading to arbitrary omissions.

A citation index will, for example, disregard the large number of times the term Feulgen is used, without bibliography and yet with precise meaning. Lead citrate staining is mostly mentioned with a citation; uranyl acetate, used for the same purpose and perhaps reported in the same sentence, without one. Citation practice for embedding media is varied and not always apt. Fixation with glutaraldehyde ranked frequent citation for a time; that with osmium tetroxide rarely does unless particular buffers are added. Because these techniques rarely form "key words", there is no automatic check on this selectivity.

This does not deny that the authors frequently cited are important and welcome influences in several fields. An appreciation of the state of their fields, however, and of their influence on them, will escape index researchers content with the objectivity gained by not reading the literature.

Yours faithfully,

P. T. P. OLIVER

Institute of Genetics,
University of Glasgow,
Church Street, Glasgow W1.

Announcements

University News

Professor Jack Diamond, Beyer professor of mechanical engineering, will succeed **Professor S. G. F. Brandon** as a pro-vice-chancellor of the **University of Manchester** in October. **Professor R. A. C. Oliver** is retiring from the Sarah Fielden chair of education and will be succeeded by **Professor Frank Musgrove**, University of Bradford.

Mr John B. Wilkinson, head of the Unilever Research Laboratory, Isleworth, has been appointed visiting professor in the Department of Chemistry, **University of Surrey**.

Professor Peter V. Hobbs has been appointed professor of atmospheric sciences in the **University of Washington**, Seattle.

Appointments

Lord Nelson of Stafford, chairman of the General Electric and English Electric companies, has been elected president of the **Institution of Electrical Engineers** for 1970-71.

Mr F. W. Dunning has been appointed curator of the **Museum of Practical Geology**, South Kensington, in succession to **Mr A. J. Butler**, who is retiring from the office in order to undertake a special assignment in the Institute of Geological Sciences.

Dr Stevenson Buchan will retire as deputy director of the **Institute of Geological Sciences**, South Kensington, in April 1971. He will be succeeded by **Dr A. W. Woodland**, assistant director, Northern Region England and Wales, and head of the Leeds office of the Institute.

Miscellaneous

Professor R. R. Haering of Simon Fraser University has been awarded the **Herzberg medal** of the Canadian Association of Physicists.

Mr S. W. K. Morgan, **Dr S. E. Woods**, **Mr J. Lumsden**, **Mr B. G. Perry** and the late **Mr L. J. Derham**, of the Imperial Smelting Corporation Ltd, Avonmouth, have won the **Royal Society Mullard award** for 1970 for their outstanding contributions to the concept and development of the zinc blast furnace. The award, which consists of a gold medal and a prize of £1,000, is made annually for an advance in science, engineering or technology which has in the past ten years led directly to national prosperity in the United Kingdom.

- * The Council of **The Royal Society** has awarded the following fellowships, tenable for two years from October 1: **Mr and Mrs John Jaffe donation research fellowship**, to **Dr A. E. Hill** to study the molecular assembly of an ion pump at the Hebrew University, Jerusalem, and the University of Cambridge; **Pickering research fellowship**, to **Dr R. K. Thomas** to continue his work on infrared and photoelectron spectroscopy at the University of Oxford; **Stothert research fellowships**, to **Mr P. R. Cook** to work on genetic activity in fused cells at the University of Oxford, and to **Dr G. D. Lindsay** to undertake chemical modification studies on insulin at the University of Sussex. The Sorby Research Fund Committee has awarded the **Sorby research fellowship** to **Dr Bridget I. Baker** to study the functions and control of teleost pituitary secretions at the University of Sheffield.

ERRATUM. In the review, "Skyie-Influences", by N. W. Pirie (*Nature*, **227**, 525; 1970), the word "courses" in line 20 of the first paragraph should read "sources", and the word "prebiotic" in line 14 of the second paragraph should read "probiotic".

International Meetings

September 2-4, **Man-Computer Interaction**, Teddington (Dr Christopher Evans, Conference Organizing Committee, Division of Computer Science, National Physical Laboratory, Teddington, Middlesex).

September 11, **Land Management in the Seventies: Concepts and Models**, Seattle (Land Management Conference, Battelle Seattle Research Center, 4000 NE 41st Street, Seattle, Washington 98105, USA).

September 16-October 24, **Plastics at the Design Centre**, London (The Information Officer, British Plastics Federation, 47 Piccadilly, London, W1V 0DN).

September 20-25, **International Federation of Societies of Cosmetic Chemists Conference**, Barcelona (The General Secretary, International Federation of Societies of Cosmetic Chemists, 56 Kingsway, London WC2).

September 22-24, **Critical Factors in the Application of Diesel Engines**, Southampton (Mrs M. Wyatt, Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1).

September 28, **The Theory, Design and Applications of the Optics Technology Inc. Modulation Transfer Function Analyser**, London (Techmation Limited, 58 Edgware Way, Edgware, Middlesex HA8 8JP).

October 6, **New Developments in Lanolin and its Derivatives**, London (The General Secretary, Society of Cosmetic Chemists of Great Britain, 56 Kingsway, London WC2).

October 12-16, **Transports and Communications**, Genoa (Istituto Internazionale delle Comunicazioni, Viale Brigate Partigiane 18, 16129 Genoa, Italy).

October 18-21, **Canadian Chemical Engineering Conference**, Sarnia (Canadian Society for Chemical Engineering, 151 Slater Street, Suite 906, Ottawa 4, Ontario, Canada).

October 20-22, **Electricity Distribution**, Edinburgh (Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

October 28-29, **Hormonal Regulation of Protein Synthesis in the Liver**, Blindern (Per O. Seglen, Zoologisk Laboratorium, Universitet, Box 1050, Oslo 3, Norway).

December 6-11, **Air Pollution**, Washington (Professor A. C. Stern, Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, PO Box 630, Chapel Hill, North Carolina 27514, USA).

March 9-14, 1971, **The Exploitation of the Oceans**, Bordeaux (Oceanexpo, 8 rue de la Michodière, 75-Paris-2, France).

March 16-18, 1971, **Electrical Safety in Hazardous Environments**, London (Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London, WC2R 0BL).

March 20-April 2, 1971, **Datafair 71**, Nottingham (Datafair 71 Conference Office, The British Computer Society, 29 Portland Place, London W1).

March 30-April 1, 1971, **Use of Electricity in Process Engineering and Petroleum Technology**, Newcastle upon Tyne (Dr A. Winward, Department of Chemical Engineering, University of Newcastle upon Tyne, Merz Court, Claremont Road, Newcastle upon Tyne NE1 7RU).

Sabbatical Itinerants

From the issue of September 5, entries of this kind will appear among the classified advertisements and will be charged for accordingly. Copy should be addressed to T. G. Scott and Son Limited, 1 Clement's Inn, London WC2A 2ED

Wanted: Guggenheim Fellow wishes to rent furnished 3-4 bedroom, centrally heated house in central London from mid-November 1970 to approximately June 1971. Excellent London and Oxford references as well as US ones if needed. Please contact Dr J. A. Stockdale, Health Physics Division, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830, USA.

British Diary

Thursday, August 27

Dynamical Aspects of Synoptic Meteorology; and Forest Meteorology
(two-day summer meeting) at the University of Edinburgh.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 266, No. 1179 (16 July 1970): Spatially Periodic Dynamos. By G. O. Roberts. Pp. 535-558. 12s; \$1.55. Vol. 266, No. 1180 (16 July 1970): Short-Range Order, Thermal Vibration and Expansion, and other Properties of Pseudosymmetric and Mixed Crystals of small Organic Molecules. By H. D. Flack and A. M. Glazer. Pp. 559-642+plates 10-15. (London: The Royal Society, 1970.) [207]
- Ministry of Technology. Safety in Mines Research 1969: Forty-Eighth Annual Report of the Safety in Mines Research Establishment. Pp. 94+4 plates. (London: HMSO, 1970.) 9s (45p) net. [207]
- List of University Institutions in the Commonwealth. Pp. 14. (London: The Association of Commonwealth Universities, 1970.) [207]
- Bulletin of the British Museum (Natural History). Zoology. Vol. 19, No. 7: A Review of the South American Iguanid Lizard Genus *Plica*. By Richard Etheridge. Pp. 235-256. 15s. Vol. 19, No. 8: On the Genus *Lycophora* and Its Relationship with the Family Hiodontidae. (Pisces, Osteoglossomorpha). By P. H. Greenwood. Pp. 257-285. 20s. Vol. 19, No. 9: A Redescription of the Species of *Eupolybothrus* Verhoeff S.Str. Preserved in the British Museum (Natural History) and the Hope Department of Zoology, Oxford (Chilopoda, Lithobiomorpha). By E. H. Eason. Pp. 287-310. 17s. (London: British Museum (Natural History), 1970.) [207]
- Aspects of Infective Drug Resistance. (Report of the Proceedings of the Symposium at the Royal Society of Medicine, 19 January 1970, sponsored by Cyanamid of Great Britain, Ltd.) Edited by Dr A. T. Mennie. Pp. 69. (London: PMP Services, Ltd., 1970. Distributed by PMP Services, 5 Delves Avenue, Tunbridge Wells, Kent.) [207]
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Is Supersonic Transport worth the Noise?

THERE is now a serious danger that intemperate expressions of opinion about the desirability or otherwise of supersonic commercial aircraft will seriously complicate the process of deciding what use to make of these machines. Senator William Proxmire's outburst in Washington last week (see page 884) is, for example, a splendid illustration of how over-ardent advocacy can destroy its own objectives. Unhappily for all concerned, there is bound to be the greatest difficulty in striking an accurate balance between the advantages and the disadvantages of technical projects as novel and as expensive as the two enterprises now well under way on both sides of the Atlantic. The United States Government, at the urging of Congress, has soberly acknowledged the importance but also the difficulty of the task by setting up an organization to which both NASA and the Federal Aviation Agency will contribute manpower and ideas so as to make a better cost-benefit analysis of the SST project than any that exists so far, and it would have been a great help if Senator Proxmire could have toned down the language in his report at least until the first findings of the working group are available, most probably in a preliminary form in September and more completely by the end of the year. Certainly he would have done a greater public service if he had been able to carry the dispute about the SST outside the narrow field within which it has so far been artificially and unhelpfully confined.

Arguments about the likely cost of the Concorde and the SST which tend to show that both projects are unlikely to make a profit for the governments concerned and may even cause permanent economic damage to the aircraft manufacturers as well as by now so familiar as to be arid. For whatever may be said about the balance of cost and commercial benefit, the sums of money which are involved are small by the standards on which most governments operate. In the United States, for example, the total cost of the SST programme is merely four or five per cent of the cost of the Apollo programme. And even the French and British governments between them will not be seriously embarrassed by the money that is actually spent on building and operating the two prototypes of the Concorde, now at the threshold of the trials intended to show whether the design performs sufficiently well to allow the aircraft to fly the Atlantic without having to leave its passengers behind.

The way in which skilled people have been employed on the two projects when there are other tasks that might have been attempted is a more serious objection to present plans, but so long as all three governments are for practical purposes committed to continuing support for their domestic aircraft industries, the people concerned may just as well be used for building super-

sonic transports as for other kinds of flying machines. In other words, even if Mr Proxmire is right in saying that governments will find that their investment is not recoverable, this will be neither a great surprise nor an overwhelming tragedy.

But will the supersonic aircraft ever be commercially viable? This is a more difficult question to decide. Confident predictions one way or the other are at this stage mistaken, for the true test of the commercial viability of these projects is not whether the manufacturers can sell enough copies to pay off their tooling costs but whether the airlines which buy the machines can then operate them commercially without further rigging the fare structure in such a way as to impose unjustifiable levies on air travellers. The chances are that both the supersonic aircraft being developed in the West will somehow surmount the first of these hurdles. If the Concorde is a technical success, there is every likelihood that the British and French governments will find themselves supporting the purchase by their nationalized carriers of enough copies of the Concorde for other competing airlines to be forced to follow suit. Then, by the late seventies when the Boeing aircraft comes into service, the commercial advantages of that larger and faster machine will probably ensure that most of the airlines then equipped with the Concorde will switch to the SST. The result will be that airlines will find themselves having to invest in new equipment at a rate exceeding \$500 million a year for a decade or more to come. The outcome could be an increase in the cost of air transport and not the decrease that progress should bring. The spate of re-equipment in the past few years has already been a powerful impediment to the continuing reduction of fares. The grim possibility that the coming of the supersonic transports might drive up fares is the point to which attention should be directed.

It goes without saying that a proper concern for the nuisances that supersonic aircraft will cause should be counted fully among the boundary conditions with which commercial supersonic aircraft must conform. There is no reason why people living near airports or beneath the flight paths of supersonic aircraft should be incommoded by their arrival on the scene. Indeed, most airports are too noisy as things are, and need to be made much quieter. The coming of the supersonic aircraft should be an opportunity for improving conditions, not an excuse for letting them become worse. If it follows from this that the flight of supersonic aircraft must be restricted to routes between terminals where noise is comparatively unimportant, that is merely a restriction that airlines should be compelled to take into their calculations. In the long run, it may even be necessary to weigh the chance that

investment in the development of better aircraft engines against the cost of building entirely new airports in places where they cannot be a nuisance. This, after all, is merely what the systems analysts would say.

Noise apart, the other environmental hazards now commonly associated with the prospect of commercial supersonic aircraft are for the time being hypothetical, which is not, of course, to suggest that they can be ignored. Unhappily, however, there is now among the environmentalists a tendency to suppose that a hypothetical danger is potentially a disaster until it can be shown to be entirely negligible. One result has been a needless exaggeration of the danger that supersonic flight will so load the lower stratosphere with unaccustomed quantities of water vapour and hydrocarbon soot that the thermal balance within the atmosphere will be upset. This is a matter to which the President's *ad hoc* working group on the SST drew attention more than a year ago, and obviously it cannot lightly be dismissed. Fortunately, however, this is not an issue which can be settled only by launching fleets of supersonic aircraft into the stratosphere and waiting to see whether there is a deterioration in the climate on the surface of the Earth. As good luck will have it, the past few months have seen valuable steps towards the better understanding of the thermal balance in the atmosphere, chiefly by the use of improved instruments in Earth satellites. With wit and moderate deliberation, it should be possible to improve enormously on the present understanding of what water vapour in the stratosphere is likely to accomplish. The chances are that here as in many other questions of the influence of human activities on the atmosphere, the inertia of the system will be too great for noticeable damage to be done, but that does not make less urgent the need of a research programme directed to this end.

The implications of these considerations for the decisions that must now be made about the future of

supersonic transports on both sides of the Atlantic are clear but unexpected. In Britain and France, to be sure, the issue may be decided in the next few weeks if it turns out that the Concorde is not commercially viable for technical reasons. If that should happen, no doubt there will be a tendency to move ahead more slowly with the Boeing aircraft, which will simplify many problems. But whatever happens, governments—not merely those with taxpayers' money tied up in development programmes—should urgently consider whether the present structure of the airline business is that best designed to make full use of the technological benefits of the modern aircraft industry. As things are, with an international cartel arrangement on fares within the International Air Transport Authority (IATA), airlines are able to compete with each other only by embellishments of the services which they offer. Some airlines win customers by the sandwiches which they serve in flight. There is now a danger that the arrival of supersonic aircraft may be yet another artificial means by which airlines can compete among themselves for passengers. To be sure, the members of IATA can be relied on to agree among themselves on a surcharge for flights by supersonic aircraft, so that the pressure of competition will not be felt too severely by those airlines only barely able to stay in business as things are, but that will only mean that such advantages as there may be in supersonic flight are put even further out of reach than the working of supply and demand would ensure. What all this implies is that governments should try to ensure that vested interests in the supersonic aircraft should not exert an exaggerated influence on the pattern of air transport, and the way to do this is to replace the present rules within IATA by more flexible arrangements that will allow airlines to compete with each other not merely on service but on price. That is the common cause on which opponents and supporters of supersonic aircraft, Senator Proxmire not excluded, should conspire.

One Myth Less

AMONG the myths of modern times, none has endured so well as the belief that the power blackouts which crippled New York and much of the north-eastern United States on November 9, 1965, were followed nine months later by a surge in the birthrate in New York City. To be sure, in the middle of the silly season in 1966, it was only natural that newspapers should be eager to fan into flame reports that the numbers of births at hospitals in New York were then abnormally frequent. The *New York Times* has the doubtful distinction of having been first with the news, and Mount Sinai Hospital of all places is said to have been the hospital at which a normal day's natality of eleven was more than doubled on Monday, August 8, when there were 28 births at the hospital. Similar if less spectacular results were reported elsewhere, and various branches of the social sciences have since

been distinguished in reputation by statements such as that of the sociologist who said "the lights went out and people were left to interact with each other". Luckily the reputation of his craft has been in part redeemed by J. Richard Udrey of the School of Public Health, University of North Carolina. Mr Udrey has shown (*Demography*, 7, 325; 1970) that reports of an enhanced birthrate in New York are most probably accounted for by that part of the collective unconscious which supposes that dreadful things always happen when the lights go out.

The truth is that, as Mr Udrey shows, that the blackout in New York had no demographic consequences worth speaking of. He has compared the number of births in New York in the summer weeks of 1966 with those in earlier years. There is statistical evidence to show that 90 per cent of children con-

ceived on November 10, 1965, should have been born between June 27 and August 14 in the following year. And day-by-day records of births in New York City apparently show that in each of the first five years of the decade the numbers of births in the corresponding intervals accounted for between 13.9 and 14.1 per cent of all births in the year. As luck will have it, the same interval in 1966 accounted for 13.9 per cent of the year's total births. Comparisons of weekly averages for the first half of the sixties show that there was no significant difference between the pattern of 1966 and that of the preceding years.

Mr Udrey is realistic enough not to expect that his simple analysis will prevent all further talk of the demographic aftermath of the great blackout but that, of course, is an exceedingly passive point of view. The question he should be asking is whether power cuts could not be used deliberately, by sufficiently clever local authorities, as a means of regulating the population. For if, as it now seems, it may be possible to create among city populations the belief that there has been a massive increase in population simply by switching off the lights, may it not also be possible by switching off the lights every few days, to create among the populations of cities such as New York such a powerful conviction that the city is about to burst with people that nobody will dare bring children into the world? Is it too much to ask that Mayor John Lindsay, with all his present troubles, should be the first to try this out?

100 Years Ago



Many persons are, no doubt, under the impression that the deaf and dumb talk to each other by means of the finger alphabet; but the use of this pre-supposes a knowledge of the meaning of words and letters, which the deaf and dumb child can hardly be taught till intelligible communication has been established with it. Alphabetical speech is slow and clumsy, whereas the deaf mute speaks to his comrades as rapidly, if not as precisely, as we do by means of vocal speech. He uses a copious and expressive language of signs, indicating words and ideas by means of simple motions and gestures. This language has the advantage of being natural and universal. English, French, and German children to a great extent understand each other, and even a North American Indian would be able to talk with them all, it being a curious fact that many of the signs used by the Indian tribes are identical with those of the deaf and dumb schools of Europe; and Mr. Tylor states that a Sandwich islander and a Chinese both made themselves understood in an American deaf and dumb institution.

From *Nature*, 2, 350, September 1, 1870.

OLD WORLD

ENVIRONMENT

Message for Alarmists

"THOSE prophets of doom who predict the more bizarre kinds of human catastrophe and paint rather self-righteous pictures of scientists as irresponsible villains exploiting humanity to the point of disaster could well be doing their own (and our) cause a great disservice." With these words, Mr F. E. Ireland, Chief Inspector of Alkali Works in the UK, in the annual report of his inspectorate to the government (*106th Annual Report on Alkali, &c. Works*, 1969, HMSO, 50p), launched into an outspoken and remarkable attack on the swelling tide of alarmist utterances about the environment which he believes are founded more on emotion than on fact. The attack is remarkable both for its forthright nature and because it flies in the face of a public opinion that is well and truly roused.

One of Mr Ireland's responsibilities is to see that factories conform to air pollution regulations, and he therefore has a vested interest in seeing that the environment is cleaned up. One of his chief worries is that alarming predictions about pollution will have two undesirable consequences: there will be a flood of questions from an alarmed public which will waste the inspectorate's time, and, more important, "we may lose sight of the real dangers and dissipate our resources".

Two such prophecies which Mr Ireland singles out for attack are that carbon dioxide in the atmosphere will produce a greenhouse effect, resulting in an increase in the Earth's atmospheric temperature, causing the polar ice caps to melt and flood a great many coastal towns and cities, and that the formation of a belt of dust in the atmosphere will cut out some of the Sun's radiation, causing the Earth's temperature to drop. Both these theories are dismissed as mere speculation. There is no sign, Mr Ireland suggests, that the various physical features of the earth such as weather, temperature, position of the magnetic pole and so on, which "undergo regular long-term periodicity in fluctuation", are being disturbed by man's efforts "which are puny compared with nature's".

Mr Ireland also takes to task those who believe that the elimination of industrial sulphur dioxide from the atmosphere will signal an end to the pollution problem. "It is estimated", he says, "that man's efforts cause the emission to air of 120 million tons per year of sulphur dioxide, whereas natural causes by comparison are responsible for 600 million tons per year. Moreover, it is not the only acid-forming gas in the atmosphere." Fortunately, however, most of Mr Ireland's natural processes take place a long way from heavily populated areas where the sulphur dioxide would do much damage.

One thing which Mr Ireland makes clear is that he is as concerned as the next man that the environment should be kept as clean as possible, but what he really wants to see is a clear recognition of the tasks that lie ahead. In this regard, Mr Ireland takes the opportunity to blow his department's trumpet. British pollution control policies, he says, represent a continual advance by the workings of conscience and conciliation, so that "our achievements are

believed to have surpassed those of any other comparable industrial country". Despite this achievement, however, the chief tasks in Mr Ireland's estimation are to reduce domestic emissions, internal combustion engine exhaust gases, grit and dust and, notwithstanding natural process, sulphur dioxide.

NUCLEAR FUEL

Centrifuge Pulls in Partners

THERE may soon be two new recruits to the European centrifuge club, if proposals submitted to the Italian and Belgian governments earlier this month are taken up. Although official comment is still scarce, it seems clear that the Italian Government in particular has been attracted by the offer of joining the centrifuge scheme with Britain, West Germany and the Netherlands for the production of enriched uranium, and may soon be negotiating its way into the project.

Nuclear Safeguards

THE United Kingdom Atomic Energy Authority has now made public the steps which it has been taking in the past few years to devise instruments which can help with the automatic monitoring of the uses to which fissile material from nuclear reactors is put. The authority is one of several organizations engaged with the International Atomic Energy Agency in the development of techniques like this. Its particular interest is in the detection of the delayed neutrons which are given off after the fission of uranium or plutonium nuclei.

In one technique that has been developed at Aldermaston, 14 MeV neutrons are used for irradiated samples, usually consisting of uranium swarf pressed into pellets or plutonium fragments packed under pressure into cylindrical plastic containers. After irradiation, the proportions of the two different materials can be determined by standard radioactive techniques for the detection of delayed neutrons, six groups of which may be emitted with half lives ranging from a fifth of a second to 55 seconds.

This method works best for highly enriched materials. For the analysis of fissile materials in solution, the authority has been working on gamma absorption, either directly through the walls of piping or tubing or by the construction of special cells at suitable places in a plant. It appears that the disadvantages of using absorption through constructional piping are considerable. A specially designed absorptiometer incorporating a plastic scintillator has been found satisfactory for gamma absorption measurements and obviously has a future in various separation plants. The authority's work does, however, suggest that electrolytic methods involving the measurement of the electrolytic potentials due to particular ions in solution may in the long run be one of the more convenient techniques.

The possibility of having an enrichment plant in Italy is naturally one of the chief benefits the Italian Government could hope to reap, and the prospect seems to be enticing. The exact role of a Belgian presence in the project seems more obscure, but one body that is likely to be jubilant over the likelihood of a wider European agreement is the European Commission itself. One of the ticklish points about the centrifuge agreement signed in February was that Britain was not a member of Euratom, and that under the Euratom treaty member states have to submit to the commission any draft agreements involving non-Euratom organizations. One objection the commission may have harboured was that Britain may be in a position to bar other members of Euratom from access to the enriched uranium. If Italy and Belgium now join, even the technicality of this objection will become wafer thin.

A reminder that the enriched fuel business is not the only one with commercial attractions came last week with the announcement that the UK Atomic Energy Authority had secured a contract from the American Combustion Engineering Company for the conversion of uranium concentrate to uranium hexafluoride. The deal is estimated to be worth between £1 million and £1.5 million. The conversion to hexafluoride is a vital step in the path from rough ore to enriched fuel suitable for reactor consumption and is the stage prior to the enrichment process, whether by gaseous diffusion or by centrifuge.

The order has come at a good time for the Production Group of the AEA. The present production of enriched uranium at Capenhurst is not sufficient to keep the hexafluoride plant at Springfield, Lancashire, at full stretch, and the completion of the centrifuge, with its capacity of 200 tons of uranium a year, is still a long way off. The production group, which trades as a separate entity and would have been amalgamated with the Radiochemical Centre at Amersham as a new fuel company under the programme of the previous government, will purchase uranium concentrates from US mining companies, convert to hexafluoride at Springfield, and deliver the high purity material for use in American gaseous diffusion plants.

GERMANY

Poised for Aerospace Boom

A SUBSTANTIAL increase is expected to be announced shortly in the funds allocated by the Federal Government for projects in the German aircraft and aerospace industry. The Federal Ministry of Industry is seeking the approval of parliament for its plan to spend DM11,670 million (£1,348 million) during the five years to the end of 1974 for projects in the air and space industry, about three-quarters of which will be for military aircraft and equipment.

The Federal Government's support for space projects shows a dramatic increase. The present estimate for the five-year period to 1974 is put at DM2,624 million compared with DM1,419 million for the period 1962-69. Although the figures are still provisional, the Federal authorities seem confident that the German air and space industry will soon emerge from the brief lull occasioned by a drop in new military projects and that production will soon be rising steeply again. The turnover of the industry, which

employs nearly 50,000 people, was about DM2,000 million in 1969, although there is inevitably a fair margin of error according to the way in which the fringe areas of the industry are defined.

Military projects in which the Federal Government already has a large stake include the multi-role combat aircraft (MRCA), involving DM2,000 million, and the new trainer aircraft costing DM55 million. Among civil projects, the Federal treasury is contributing DM228 million to the development of the short-range VFW 614 aircraft and at least DM750 million to the European airbus, the A-300 B.

UNIVERSITY ENTRANCE

Oxford and Cambridge in Demand

THE demand for places at the Universities of Oxford and Cambridge shows little sign of slackening off. This year, the universities attracted more than twice as many men as they could accommodate and more than four times as many women. But, while the total number of applications for places at Oxford was slightly higher than that for 1969, the University of Cambridge attracted nearly 400 fewer applications than it did last year.

These figures, which have been published by the admissions offices at the universities (*Oxford University Gazette*, supplement to No. 3440, and *Cambridge University Reporter*, No. 4719), show that although the demand for places at Cambridge is still greater than that for places at Oxford, the traditional disparity in applications for each institution is beginning to disappear. Both institutions also continue to attract many more applicants for each place than do other universities—Dr Geoffrey Templeman, chairman of the Universities Central Council on Admissions, estimated that the number of qualified applicants who were chasing places in all British universities in 1968 outnumbered the places available by about 1.5 to 1.

Table 1. APPLICATIONS AND ACCEPTANCES FOR OXFORD AND CAMBRIDGE 1970

	Oxford		Cambridge	
	Applica- tions	Accept- ances	Applica- tions	Accept- ances
Men:				
Arts	1,465	756	1,687	828
Social Sciences	792	355	764	343
Science	1,728	813	2,451	1,222
Total	3,985	1,924	4,902	2,393
Women:				
Arts	1,041	275	1,097	168
Social Sciences	197	51	150	19
Science	554	167	629	134
Total	1,792	493	1,876	321

The educational and social background of students accepted for places at Oxford and Cambridge also differs significantly from those accepted by other universities. Fifty-six per cent of the students accepted for places at Oxford, for example, came from independent and direct grant schools, compared with 43 per cent from maintained schools, while for Cambridge the figures are 56 per cent and 41 per cent respectively. There is some indication, however,

that the share of the acceptances which go to students from independent and direct grant schools has been falling slowly during the past few years, while that for students from maintained schools is on the increase.

Oxford and Cambridge also do not seem to share the difficulty found by most universities in attracting students to study science and technology—the demand for science places among the men who applied for places next October was not significantly less than that for other subjects. Both universities also experienced a drop in the number of applicants for arts subjects, compared with applications last year, while the number of applications for places in science at Oxford increased by 300.

UNIVERSITIES

No Change in 1968

THE University Grants Committee this week published the latest pamphlet in its series of statistics of education. The statistics, although they are two years old, make interesting reading and they indicate that the broad trends in student numbers and university finance which developed during the early 1960s showed little signs of change in 1968–69 (*Statistics of Education: Universities, 1968*; HMSO, £2.40).

The total number of students engaged on full time courses at British universities in 1968–69, for example, increased by 5.7 per cent compared with the previous year to 211,485. The number of full time teaching and research staff, on the other hand, increased by only 2.8 per cent, from 25,353 in 1967–68 to 26,067 in 1968–69. This caused the staff/student ratio to increase from 7.9 : 1 to 8.1 : 1. Looked at on their own, these figures seem to indicate that the government's desire to see teaching costs reduced in the 1970s was already being realized two years ago. But the figures must be seen in the light of increases in the numbers of students and staff in the previous year. In 1967–68, the number of students increased by 8.5 per cent while the number of staff increased by 11.5 per cent; the staff/student ratio has in fact remained constant at about 8 : 1 since 1958.

The exodus from the sciences into the arts and social sciences also showed little sign of easing off in 1968–69. The proportion of students studying science based subjects, at 55.8 per cent, was 0.7 per cent lower than in the previous year. Between 1965–66 and 1968–69, the proportion of students taking arts subjects increased from 41.9 per cent to 44.2 per cent, mirroring the decline in the proportion of science-based students. By far the most striking comparison, however, is that 23 per cent of the men were studying engineering and technological subjects, while only 0.8 per cent of the women students ventured into this field.

In 1967–68—the latest year for which figures are available—the universities received £216.6 million, chiefly as a recurrent grant from the Exchequer (£157.8 million) for recurrent expenditure and a further £78.4 million for capital expenditure. Salaries swallowed up more than 40 per cent of their recurrent expenditure, and departmental expenditure met by research grants took a further 11 per cent. The total grant to the universities therefore increased by 8.4 per cent, while student numbers in that year increased by 8.5 per cent.

IRELAND

Education Renaissance in Galway

A SIGNIFICANT milestone in the 120 year history of University College, Galway—and indeed in the history of higher education in Ireland—has recently been passed. A project consisting of a two-storey science building to house departments of biochemistry, chemistry and physics linked with a four-storey library block was set in motion earlier this month when Dr M. O. Knuthail, the college president, signed a contract worth £2 million with John Sisk and Sons Limited, a local building firm.

A grant for the development of University College, Galway, was announced at the end of 1967 by the late Mr Donagh O'Malley, then Minister for Education, and it will provide eventually for a single campus of some 3,000 acres, accommodating 6,000 students. The overall cost is estimated to be £10–£12 million. The first stage of the project, the science building and library, should be completed in time for the 1973–74 session.

The University of Galway project is at least tangible evidence that Ireland is emerging from a period of stagnation in higher education. A report published earlier this year by the Organization for Economic Cooperation and Development drew attention to the slow development of higher education, and in particular to the lack of science and technology students—projections indicated that there should be about 19,000 students enrolled in Ireland's universities in 1970–71, of whom only 20 per cent are expected to study science and technology. The OECD report repeatedly pointed out that educational planning authorities have given insufficient attention to higher education during the 1960s.

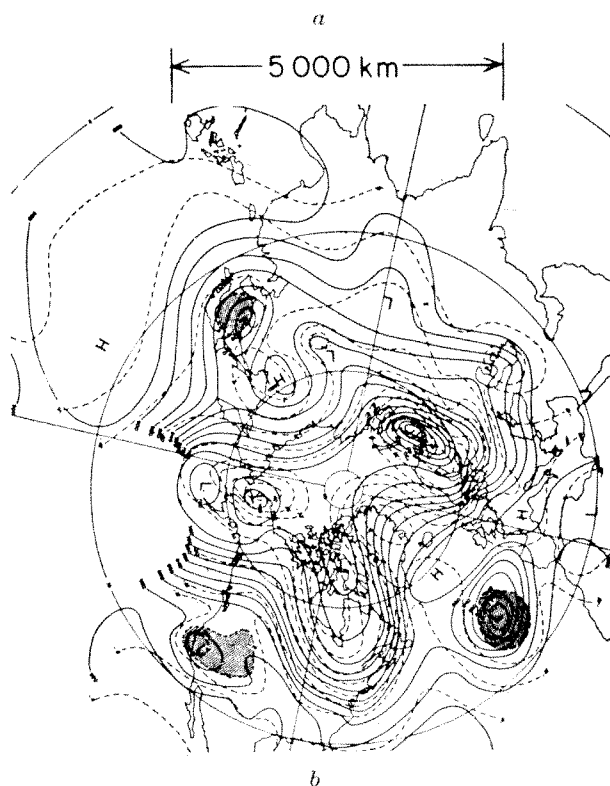
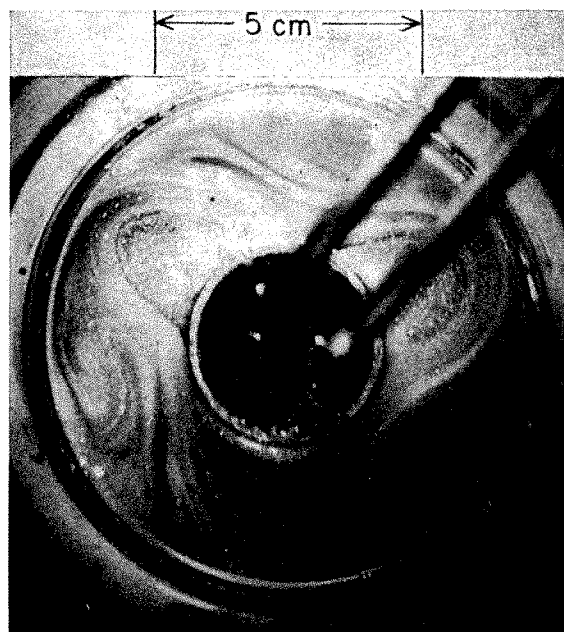
METEOROLOGY

Next Year's Forecast

RELIABLE weather forecasts for up to a week ahead are promised by the Meteorological Office as one of the benefits of the new computer it is installing next year. And, according to the report of the office for 1969 (HMSO, London, 13s), daily forecasts should include the amount and distribution of rainfall that is expected over Britain and western Europe. The computer is to be an IBM System 360/Model 195—the largest available from IBM—and as well as putting advances in mathematical modelling techniques into forecasting practice, it will be used to continue research into the evolution of large scale weather systems.

One prerequisite for better and quicker forecasting is a fast telecommunications system to deal with data from stations in Britain and overseas and with the growing quantity of information from weather satellites. Here the Meteorological Office is also bringing in more computers: the first stage of the automation of its telecommunications centre, which should be completed later this year, will establish it as a principal link in the Washington–Moscow trunk circuit of the World Weather Watch, and during the next few years the whole of the British data-gathering network will be modernized. This updating will include the setting up of an automated radiosonde system at sixteen

weather stations, and operational trials of a number of completely automated stations.



Simulating air movements in the laboratory. *a*: the flow pattern at the top of a rotating annulus of water; *b*: contours at intervals of 200 feet (—) and isotherms (---) at the 500 mb level for 0400 h on October 12, 1945. (Crown copyright. Published by permission of the Controller of HMSO.)

On the research side, laboratory models of atmospheric motion have been helping, sometimes quite graphically, to show how complex movements on a large scale can be caused. The technique that a team under Dr R. Hide has developed uses rotating cylindrical pans of water, heated at the circumference and

cooled at the centre to simulate the temperature gradient between the pole and the equator, and the patterns that are revealed by a suspended indicator such as aluminium powder can often resemble the familiar shapes of the weather map. There are, for example, disturbances which resemble cyclones and fronts—a fact which implies that the condensation of water vapour is not one of the most important factors controlling the occurrence of these phenomena in the atmosphere. If the heat source is moved around the circumference of the pan, the daily cycle of solar heating can be simulated, and the ensuing motions are likely to be helpful in understanding how the atmospheres of other planets behave. Even the apparent shearing motion at the edges of the Great Red Spot of Jupiter seems to have its parallels in the model.

ANTIBIOTICS

Swann Implemented

THE British Government is to enforce from next March the classification of antibiotics into feed antibiotics, which can be mixed with animal foods to promote better growth, and therapeutic antibiotics, which cannot be given to animals without a veterinary surgeon's prescription. This separation was the chief method for reducing the risk of transferring resistant microorganisms from animals to man recommended by the Swann committee last November (see *Nature*, **224**, 835; 1969). In deciding which antibiotics to ban from feeds the government has largely followed the views of the committee.

Penicillin, chlortetracycline and oxytetracycline thus become "therapeutic", and the retail sale or supply of feeds containing them will be unlawful except on prescription or with written veterinary authority. Tylosin, most of the sulphonamides and four nitrofurans will be available only on prescription. Here, sulphaquinoxaline and sulphanitran will, in spite of Swann, still be allowed in feeds for poultry to provide resistance to coccidia, a group of protozoans which occur as parasites in the digestive system. The government is apparently happy that the use of these two sulphonamides will not conflict seriously with the Swann criteria for feed antibiotics: that they should be of economic value, have little or no therapeutic application and not lead to the development of resistant strains of organism. Sulphanilamide will also be available without prescription, but only as a surface wound dressing for animals.

The approved feed antibiotics will then be flavomycin and virginiamycin, which are to be officially classified as such in time to be freely available before the other antibiotics are outlawed. A third feed antibiotic, zinc bacitracin, has already been passed for use in promoting growth.

RABIES

Dogs and Cats Reprieved

MR JAMES PRIOR, Minister of Agriculture, and Mr Gordon Campbell, Secretary of State for Scotland, wasted no time in accepting the chief recommendations of the interim report of the Committee of Inquiry

on Rabies (see *Nature*, **227**, 765; 1970). It took them just one week to digest the report and to announce that the complete ban on the import of domestic dogs and cats will be lifted on September 16. They have also decided that a quarantine period of six months is sufficient provided that imported animals are vaccinated twice during their stay in quarantine kennels.

One of the committee's recommendations which will not be implemented, at least for the time being, is that dogs and cats should be vaccinated before import. This recommendation, the Ministry of Agriculture suggests, requires further exploration because it would necessitate changes of procedure in the country of export. Should rabies break out in a quarantine kennel, the period of quarantine for other animals in the kennel will be extended for as long as the ministers think fit and no more animals will be admitted to the kennel concerned. This precaution is presumably dependent upon the committee of inquiry's final report on the isolation of animals inside kennels.

The 850 dogs and 150 cats which are at present in quarantine and which will have completed nine months in kennels by September 7 can look forward to an early and painless release—they will be allowed home without vaccination on that date. The remainder of the dogs and cats in quarantine kennels—about 1,100 dogs and 200 cats—will be only a little less fortunate. They will have to be vaccinated twice, and will be returned to their owners from September 30 onwards, as soon as they have completed their nine months' stay in kennels. As far as other canine and feline animals are concerned, the committee has not made up its mind whether or not the ban on their import should be lifted and the ministers have accordingly decided that the ban should stay. But any of these animals which have already served nine months in quarantine can be freed from restrictions on September 7.

AGRICULTURAL SCIENCE

Missed Targets

by our Soviet Correspondent

SOVIET agriculture is lagging behind the target proposed for it in the 1966–70 five-year plan. This was pointed out in no uncertain manner at the July plenum of the Central Committee of the CPSU, and was officially disclosed last week by F. D. Kulakov, secretary of the Central Committee, during the opening speech of the Lenin All-Union Academy of Agricultural Sciences. The Academicians of the LAAAS pointed out that the development of agriculture is inseparable from the progress of science, and that the facts presented at the July plenum call for the development of improved methods of tilling the soil, for new, high yielding strains of crops and livestock, and for new means, especially biological ones, of protecting plants. The need for new and more effective technology leading to the complete mechanization of agriculture and stock-rearing, and for "economically based recommendations" for the agricultural industries on a regional, rather than an all-union basis were also emphasized.

This meeting comes at a significant point in the history of Soviet agriculture—shortly before the

Twenty-Fourth Party Congress (now scheduled for next spring), and on the threshold of a five year plan. If the planning authorities draft their directives in accordance with the proposals which arose from the discussions at the conference, the forthcoming plan should yield results that are considerably more satisfactory than the last.

HOVERCRAFT

Hover-Over from Canada

AN overseas firm using British hovercraft patents under licence has just put its home-developed hovercraft on the English market. The specially formed company, Canahover Ltd, a subsidiary of the Ottawa Bogue Electric Manufacturing Company, is currently demonstrating the Hover-Over on the Thames and a freighter version is on view at Ontario House, Charles II Street.

The Hover-Over can also be used as a two-seater sports vehicle or as an amphibious runabout. The single-seat freighter version has 600 lb. of payload available in a well behind the driver's seat and it also has a future as a seismic or general survey craft, while the two-seater could be used for waterway patrolling. The price quoted is between £2,300 and £3,000—there is some uncertainty about carriage costs from Canada—and there is no import tax.

The craft is 16 feet by 7½ feet with a 16 inch skirt of all-Canadian design. It is powered by three Rockwell 2-stroke air-cooled engines originally developed for the 'sno-mobiles' now so popular during the Canadian winter. It is very robust and will be able, it is claimed, to travel over water, mud, snow and sand. A great advantage is that this engine has a very narrow range of operating temperature—in previous small hovercraft most of the engine trouble encountered has been due to the cold-water dousing of an engine running very hot. Altogether \$2 million (Canadian) has been invested in modifying the engine over a period of two and a half years.

The Hover-Over can reach 50 knots under smooth conditions and cruises at 35 knots. It is said to be very economical of fuel. Two hundred craft have been sold in North America since it was put on the market there last spring.

Its most direct competitor on the English market is the Hoverhawk made by the East Anglian firm of Hover-Air. This has been powered in the past by a two-stroke motor-cycle engine. From the current demonstrations the Hover-Over seems marginally more manoeuvrable.

The sales drive for the Hover-Over has started in London because of its central position for marketing both in Europe and the Middle East. The company has its eye in particular on Central and Eastern European waterways, and on the oil industry's activities. All the oil companies, it is pointed out, have London offices.

Mr Griffen, Hover-Air's general manager, considers that the British hovercraft industry will have much bigger earnings in North America through selling licences and collecting royalties from North American built vehicles than in direct sales. Last winter a contract for 4,000 Hoverhawks was said to have been placed by Canadian interests.

Miscellaneous Intelligence

THE irony of the new appointment as head of Space Division at the Ministry of Technology on the translation of Mr Richard H. W. Bullock to a deputy-secretaryship could hardly be more complete. It goes to Mr A. Goodson who for the past three years has been on loan to ELDO, and what is more, as Director-General of Financial Affairs. The main thrust of the policy that Mr Bullock has been responsible for pursuing was to prove the non-credibility of ELDO and to withdraw Britain's financial support from it. Can this appointment be taken as the climax of the policy's success?

THE story of how an ornithological fraud of almost Piltown dimensions was foisted on British scientists in the 1900s with effects to this day is revealed in the current issue of *British Birds*. 595 rare bird species that had never before been recorded in Britain were reported over a period of years by a Sussex taxidermist called Bristow. He claimed to have shot them on the Hastings coast and in spite of the extraordinary degree of coincidence involved, the 595 "Hastings Rarities" were accepted by the establishment. A number of them were even included in the 1952 definitive *Check List of the Birds of Great Britain and Ireland* on Bristow's evidence alone.

Some British bird men were increasingly suspicious of the Rarities but could not account for how Bristow had obtained fresh specimens of foreign birds. Robert Coombes now tells how he chanced to meet the man who smuggled in the birds from the Persian Gulf, the Baltic and the Mediterranean before the First World War. He was a ship's steward called Parkman, and, on a commission from his brother in England, he shot or bought wild birds at his ports of call and kept them on ice on the voyage home. There he handed them over to his brother who, he knew, disposed of them at Hastings "to a man called Bristow".

Almost equally extraordinary is the time it has taken to get the record straight. Mr Coombes learnt about the bird smuggling route in 1939, when he was importing some geese specimens from Persia, which Parkman, still on the Middle East run, cared for en route. In 1947 when Coombes was an officer of the British Ornithologists Union he mentioned the matter to the then President, Sir Norman Kinnear, who told him to keep quiet. This he has reluctantly done for another 23 years.

THE Soviet journal *Khimia I Zhizn* claims that Soviet biologists have discovered that human beings exhale a variety of obnoxious substances, including "hydrocarbons, alcohols, ammonia, formic and acetic acid, formaldehyde and acetone". It is no wonder that all the talk about cleaning up the environment seems to have had little effect.

THE prize for the most amusing scene at the meeting of the International Astronomy Union held in Brighton last week must surely go to the opening celebrations. Much to the embarrassment of the British astronomers, the band of the Coldstream Guards launched into "Rule Britannia", and about 1,000 astronomers from all over the world joined in the chorus. The astronomers were later treated to a rendering of "Twinkle, Twinkle, Little Star".

Pulsars Explained, Craters Named at IAU

from our Special Correspondent

Brighton, August 25

It might have been expected that the general assembly of the International Astronomical Union that opened in Brighton last week would have been dominated by pulsars. After all, they were unknown at the last general assembly three years ago. But the IAU Symposium held at Jodrell Bank at the beginning of August seems to have preempted the debate (for a report see *Nature*, **227**, 651; 1970). That is without counting the resourceful Professor Vitaly L. Ginzburg, of the Lebedev Physical Institute (Moscow), however. Professor Ginzburg was not at the Jodrell Bank meeting, and he arrived at Brighton with a theory of the pulsar radiation mechanism based on the maser emission process to explain the extraordinarily high brightness temperatures of 10^{23} K that are recorded. "Maser mechanisms", he says, "are quite capable of explaining all the peculiarities of the radio emission of pulsars." People were saying, however, that if Professor Ginzburg had seen the polarization measurements that were presented at Jodrell Bank he would not have been so confident.

Now that nobody is doubting that pulsars are rapidly rotating neutron stars, the discussion has centred on the radio emission process. By comparison, the questions of how the neutron stars are formed, and what they are like inside, are receiving less attention. The reason seems to be the feeling that not enough is known about the physics of matter at the densities of 10^{12} grams per cubic centimetre, and more, that are believed to exist inside neutron stars. But the observational material that has been built up over the past two and a half years on the radio, optical and X-ray pulses is for the moment just as hard to disentangle. Most attention is being given to two facets that people intuitively believe must hold the key. First, the way that the angle of the linear polarization often rotates throughout the duration of a pulse at a rate that is characteristic for each pulsar is felt to be important. Second, at least five of the fifty-five pulsars so far recorded seem to contain a system of weaker pulsations at rates of 10 to 100 per second superimposed on the main pulses. T. W. Cole from Sir Martin Ryle's group at Cambridge reported that in the case of CP 0808, the speed at which these subpulses drift through the main pulse follows a sawtooth pattern. But so far nobody has been able to work the polarization results and the subpulses into a theory of pulsar emission that their colleagues will accept.

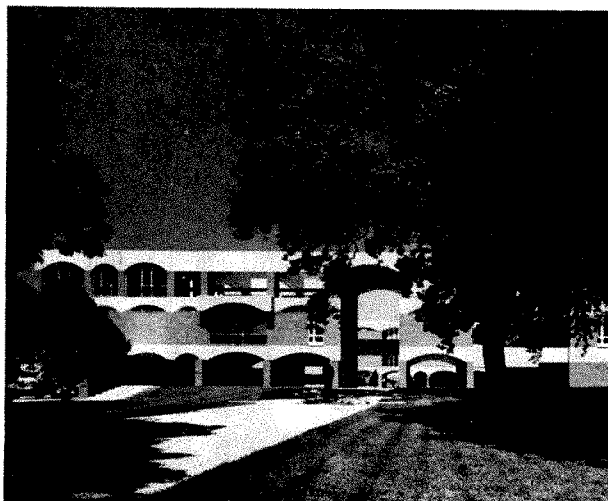
It was inevitable that the second talking point at Brighton should have been the new data about the Moon. Again, with the Apollo geological and seismological data entirely new since the last general assembly in Prague, there has been a lot to talk about. Yet it is odd that what has captured people's imaginations has been the naming of the craters on the far side. A working group of four to select the names was appointed at the Prague meeting, and their list was expected to be approved on the final day at Brighton. Professor Donald Menzel (Harvard College Observatory), chairman of the group, described the work as

"very interesting but arduous labour". His colleagues were Professor M. Minnaert (Utrecht), Professor B. Levin (Moscow) and Professor A. Dollfus (Paris).

They had wanted the list to be as international as possible and national academies of science were asked to submit names. The 1,200 names received were then pared down to a list of 513 that the group felt were the most distinguished, representational and truly international. One idea which the group was keen on was to assign the names in alphabetical order from the north to the south pole. Aesthetic sense prevailed, fortunately, and instead the names were divided among five categories of distinction, and the craters among five categories of size. Names were assigned to craters in the corresponding distinction category by drawing lots.

Forty-nine British people are listed, chiefly scientists but including Geoffrey Chaucer and H. G. Wells. It was a nice touch that there should be a crater named after Sydney Chapman who died earlier this year. Other British names on the list include Sir Edward Appleton, Robert Boyle, William Bragg, Sir John Cockcroft, Sir Alexander Fleming, Oliver Heaviside and Sir Harold Spencer Jones. But the New Zealander Lord Rutherford had to be omitted because of the possibility of confusion with a crater Rutherford on the near side. The same happened to Born because of confusion with Bohr.

A dozen names of living people have also been assigned craters, setting something of a precedent. The three Apollo 11 astronauts have been given small craters a few kilometres across near the landing site in Mare Tranquillitatis, and the Apollo 8 astronauts, the first to fly behind the Moon, have craters on the far side. To redress the balance six living Soviet cosmonauts have been named. The names of the American astronauts killed in the launch pad fire have been given to small craters near a far side feature to be called



University of Sussex, where part of the assembly is taking place. Designed by Sir Basil Spence, Bonnington and Collins.

Apollo and the names of dead Soviet cosmonauts are given to features near Mare Moscovense. But the name of Yuri Gagarin, killed in an air accident in 1968, has been given to one of the larger features on the far side.

As *Nature* went to press the list of names had not so far caused any international ructions. Objections had been raised to about half a dozen names on the list by the IAU Commission on the Moon, but these it seems were trivial points rising, for example, from possible confusion when names were assigned to small craters within larger named ones. In these cases the working group agreed to move the names to other locations.

Debate about the scientific data on the Moon that the Apollo programme has produced seems to be centring on the age measurements for the lunar material, and on the seismic data. Ages as great as 4.5×10^9 years have been assigned to some of the samples, and the debate appears to be narrowing down to a discussion of precisely what are the zero points of the conventional dating techniques. The seismologists, on the other hand, are deciphering the 250 natural events that have so far been recorded, and they seem to have been remarkably successful. It has been possible to identify a class of moonquakes which occur when the Moon is at perigee, presumably due to tidal forces in the interior, and to distinguish these from events caused by meteorite impacts. The meteorite events have been attributed to meteorite masses of a few kilograms, and the rate nicely fits the Hawkin's meteorite distribution curve. But the burning question which Professor M. Ewing (Columbia), one of the lunar seismologists, was at a loss to explain was why the traces from a few of the events are an almost perfect match. "I have never seen terrestrial earthquakes that match so well", Professor Ewing said.

Secrecy and Little Green Men

AT Brighton last week Professor V. L. Ginzburg, of the Lebedev Physical Institute, met Dr A. Hewish of the Mullard Radio Astronomy Observatory for the first time since the discovery of pulsars was announced in *Nature* two and a half years ago (217, 709; 1968). Professor Ginzburg had been staying with Dr Hewish at Cambridge while the Cambridge group was grappling with the problem of the origin of the regularly pulsing signals. But Ginzburg, now one of the foremost Soviet theorists on the pulsar problem, was not told of the discovery. The omission rankled, Ginzburg admits, when he returned to Moscow and saw the first report of pulsars in *Nature*. But at Brighton last week Ginzburg agreed with Hewish that the Cambridge group were right to keep the signals a secret until the experiments to check whether they were coming from extra-terrestrial life had been completed. Dr Hewish and the Cambridge group are forgiven, Professor Ginzburg says, now that he (Ginzburg) realizes that the British astronomy community was not party to the secret either. Dr Hewish explained that if the discovery of pulsars had been reported any earlier the Cambridge observatory would have been overrun by the press, making work impossible.

RADIO ASTRONOMY

Case of the Unidentified Line

ANOTHER addition to the growing list of molecular species that have been identified in interstellar space is expected soon. L. E. Snyder and D. Buhl reported to the IAU meeting on interstellar molecules on Monday that a new line has been detected that is so far unidentified. The line has a rest frequency of $89.190 \text{ GHz} \pm 2 \text{ MHz}$ and has been seen in four, probably five, sources. It was first picked up while Snyder's group was recording a line of HCN using the 36 ft telescope of the National Radio Astronomy Observatory at Kitt Peak, Arizona. The unidentified signal appeared in the upper sideband while the line due to molecules of HCN containing the carbon isotope ^{13}C was being observed in the lower sideband. Snyder said that he is "fairly convinced" that the line is molecular. And it is unlikely to be a Doppler-shifted HCN line—that would require a radial velocity of 1,690 km per second, an order of magnitude greater than the velocities that have been measured in interstellar clouds. The unknown molecule has been named X-ogen by Snyder and his colleagues and it has been recognized in W3, Orion A, Sagittarius A, W51, and probably in L134.

It was only two months ago that Snyder's group reported the detection of interstellar HCN. So far this molecule has been detected at 88.6 GHz (3.4 mm) in six sources, W49, DR21, Orion A, W3, W51 and Sagittarius A. It is also pleasing that the line corresponding to substitution by the isotope ^{13}C has been detected—the line that was being observed when X-ogen was discovered. The abundance ratio $^{13}\text{C}/^{12}\text{C}$, significant for theories of nucleogenesis, can then be determined.

The list of molecules that have been detected in the past year or so makes interstellar space sound like an organic chemistry laboratory. Professor C. H. Townes (Berkeley), who as much as anybody inspired the search for these molecules, pointed out at the meeting that a lot of hard work had been unsuccessful. Although ammonia, formaldehyde, and even cyanacetylene (HC_3N) have been detected, some simple molecules that might have been expected to be present because of their stability have not been found, for example, $\text{H}_2\text{C}_2\text{O}$. SiO , SO and H_2CO_2 have been looked for reasonably hard, but not found. One molecule which Townes predicted would be detected before long was NO. But he admitted that he could give no convincing reason why the molecules should be present to the extent that they are, although he agreed that conditions in the dark clouds where the molecules are found are likely to be the most favourable.

It was obvious from the meeting that the molecules are a nice source of surprises. Why, for example, should the abundance ratios $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ obtained from measurements of the isotopic variations of formaldehyde (H_2CO) disagree with the ratios observed in stars, and instead be more like terrestrial abundance ratios? What makes the H_2O line detectable, when work with very long base lines shows that some of the sources are less than one astronomical unit across? Amplification by the maser process is assumed. And why is the formaldehyde line seen in absorption when there is no continuum source in the background apart from the 3K isotropic radiation? In this case, some kind of maser process in reverse is suspected.

NEW WORLD

All Change at the Top

from our Washington Staff

DR LEE DUBRIDGE, Science Adviser to the President for the past few years, startled the establishment last Thursday with the announcement of his resignation from the end of August. He will be succeeded by Dr Edward E. David, a computer engineer from the Bell Telephone Laboratories. Dr DuBridge's departure from the White House will be accompanied by other important changes. At the National Science Foundation, the post of deputy director has at last been filled, again by an engineer—Professor Raymond L. Bisplinghoff, dean of the school of engineering at MIT. In the past few days it has become known that the successor to Representative Emilio Q. Daddario, chairman of the Subcommittee on Science, Research and Development, in the House of Representatives, will be Mr John W. Davies, the most senior of the six Democrats on the subcommittee.

The departure of Dr DuBridge will have the most immediate effect. He said last week that he had always had "a horror of staying at any job past reasonable retirement age". There is no doubt of the affection with which he is held in Washington, where he has been highly respected. It is also widely appreciated that the best time in which to be Science Adviser to the President is when expenditure on research and development is growing quickly, for then there is a good chance that the extra funds will be supposed to have been conjured up by the Science Adviser himself. From this point of view, Dr DuBridge has been unlucky, but his spell in office has also been marred by the muddle over the effects of the Mansfield Amendment on the pattern of federal research expenditure—effects that could have been anticipated. At the end of last week, Senator Mansfield went out of his way to emphasise that he had no personal quarrel with Dr DuBridge on this score.

Dr David is a Bell Labs man to the core. He moved there from MIT in 1950 and worked for more than a decade on underwater acoustics. Since then he has been engaged on computing science and has also played an important part in developing the high school curriculum intended to introduce young people to a sense of the importance of technology.

The appointment of Professor Bisplinghoff as deputy director of the NSF also raises new problems, for the post that he will occupy was only created two years ago and has not previously been filled. In an attempt to strengthen the foundation's hand in the Administration, Congress argued that it should have a deputy director and four assistant directors all of them appointed by the President. The four assistant directors

are already at work (see *Nature*, **226**, 105; 1970) and no doubt the deputy director would also by now have been at work for some time if the first choice for the post, Professor George Hammond, of the California Institute of Technology, had not blotted his copybook in June by speaking out against the Cambodian operation. There remains, however, some confusion about the precise function of the men now serving as assistants and deputy directors. With the National Science Board brooding over the foundation's work, does it really need six highly paid administrators to spend a mere \$500 million?

Dr Bisplinghoff has a NASA background, having been associate administrator for advanced research and technology from 1963 to 1965 and special assistant to the then administrator, Mr James Webb, for a further two years. More recently, Dr Bisplinghoff has been the chairman of a working party intended to advise the Administration on supersonic air transports. He is known to take the line that the SST would be a fine thing if its noise could be kept within bounds.

In the long run, the appointment of Representative Davies as chairman of the subcommittee on Science, Research and Development may turn out to be the most important of all the changes. Mr Daddario has for several years exercised a powerful influence on science policy, chiefly by making himself one of the few people

No Budget Yet

THE National Science Foundation is one of several agencies whose budgets for the current year have been snatched away by the President's veto on August 11 of the independent offices. On the same date, President Nixon also vetoed appropriations for the Office of Education. Both bills between them included roughly \$1,000 million for work not originally spelled out in the Administration's budget—\$453 million extra for education and \$451 million extra for the independent agencies. Although the National Science Foundation stood to gain \$9.6 million above the Administration's request for \$513 million, what seems to have stuck in the Administration's throat is the extra \$300 million appropriated by Congress for urban development. The next step will be for Congress to devise a more acceptable bill and there is a chance that this will find its way through Congress in the next few weeks.

in Congress who understood what the Administration was doing. Towards the end of his reign—Mr Daddario is almost certain to be elected governor of Connecticut in November—he has been influential enough to develop the legislation within which the NSF now operates. In the past few months, Mr Daddario has also lent his enthusiasm to doubtful projects such as that for setting up in Congress what he calls an Office for Technology Assessment.

Representative Davies is known to share Mr Daddario's enthusiasm for a stronger National Science Foundation. He is an amateur astronomer and holds a pilot's licence. His succession will not finally be assured until after the election in November, although there seems no doubt that he will be returned by his district in Georgia.

CBW

Geneva Protocol at Last

WITHIN hours of the scuttling of the old Liberty ship LeBaron Russell Briggs with more than 10,000 disused nerve gas rockets on board, President Nixon last Thursday sent to Congress his proposal that the Geneva Protocol on the use of chemical and biological weapons should be ratified. This step has been on the cards for the best part of nine months, ever since the Administration conceded that the case for including these materials in the arsenals of the twentieth century had become irrelevant. By all accounts, the delay has been brought about partly by the need to work out a policy on the use of tear gases and defoliants, materials used not merely as agents for the restoration of civil order but also as military weapons of a kind, as in Vietnam. The compromise which seems now to have been arrived at is that the United States Government has accompanied support for the Geneva Protocol with a declaration that tear gases (especially CS gas) and defoliants will be excluded from the provisions of the protocol, but that these materials will not be used without express permission from the White House after the ending of the Vietnam War. It remains to be seen whether those in Congress who are asking for a prohibition of the use of military appropriations for chemical defoliants will be able to dislodge this carefully calculated position.

The Geneva Protocol, which prohibits the "use in war" of chemical and bacteriological weapons, was first negotiated within the League of Nations in 1925 and has been ratified by 85 nations. The unwillingness of the United States to ratify the declaration has always been regarded as one of its more obvious defects. The President's message to Congress last week reserves for the United States the right to use chemical weapons if these should be used in war by either an ally or an adversary. Similar reservations have been made by many of the other nations which have ratified the protocol, the Soviet Union, the United Kingdom and France included. On the other hand, the recommendation by the Administration to Congress goes further than the position of many of the other countries which have ratified the protocol in suggesting that bacteriological weapons will never be used, whatever other countries may do. In an accompanying document, Secretary of State Rogers also makes it clear that the

United States Government considers that toxins, however manufactured, will be considered as biological weapons and not chemical weapons. In this and other ways, the position of the United States on chemical and biological weapons has now been transformed within the short space of a year. Controversy remains about the use of chemical agents in Vietnam, but the fact that the new position seems to have been reached after a hard-headed appraisal of the military inutility of these materials as weapons is not considered a disgrace.

SUPERSONIC TRANSPORT

Senator Proxmire Takes Off

THE Subcommittee on Economy in Government, in the person of Senator William Proxmire, last week launched an attack on supersonic aircraft, Anglo-French and American alike, so carefully contrived to prevent them reaching commercial service that it may have the effect of rallying support in their favour. One of the weaknesses of Senator Proxmire's position is that his subcommittee's report on the supersonic aircraft project has already been repudiated by two of the nine members—Senators John Sparkman and Stuart Symington. Another is that the witnesses who gave evidence at the hearings on which the report was based were notable for the absence of staunch defenders of the SST. (Officials of the Boeing Company, now building the supersonic prototypes, declined to appear.) In a dissenting statement accompanying the Proxmire report, one member of the subcommittee, Representative Clarence J. Brown, says that "if the joint Economic Committee had been advising Queen Isabella, we would still be in Barcelona waiting to prove the world round before daring the Atlantic".

The effectiveness of Senator Proxmire's attack on the SST project should be known in the next few weeks, when the Senate is due finally to vote on the proposal to include \$290 million for the development of prototypes in the budget appropriation for the Department of Transportation. There seems very little doubt that the vote this year will be closer than in the past. In the past few weeks, there have also been signs that the Administration is more ambivalent than ever about the virtues of the SST, chiefly because of the difficulty of estimating the effects of supersonic aircraft on the environment. In circumstances like these, external developments could have an important influence on the United States programme—if the performance of the Concorde in its impending series of trials should turn out to be far below specification, for example, Congress would probably decide to save \$290 million.

The subcommittee's case against the SST is that the social costs outweigh the potential benefits of supersonic civil aircraft, that the project is neither sufficiently radical to carry the technology of flight further forward nor so prestigious as to add to the international reputation of the United States, and that the Anglo-French Concorde project is not a serious commercial threat to the United States aircraft industry.

There has been a steady stream of argument in the past few years about the effect of the SST project on the United States economy, internally and externally. The Proxmire subcommittee now comes out firmly with the view that the effect of the project on the level of

employment will be unimportant—at the public inquiry during May, the chairman and one of his witnesses, Representative Henry Ruess, were fully agreed that a project costing \$1.3 million (up to the end of the prototype phase in 1973) and providing some 20,000 jobs at the Boeing plant at Seattle would be a needlessly expensive way of mounting a public works programme. Mr Ruess was the first of those giving evidence to the subcommittee to ask that the Federal Aviation Agency should publish the regulations on aircraft noise that will have to be satisfied by supersonic aircraft even before the first of the supersonic transports has flown. If this were done, Mr Ruess argued in May, “the rules . . . will ban the Concorde from this country. With the Concorde effectively banned from the profitable transatlantic market, it could no longer be considered a threat to the US aircraft industry or to the US balance of payments. And with the Concorde out of the competitive picture, the way is clear to call a halt to the SST.” Representative Ruess went on to say that the British and French governments had both been given a chance, a year ago, to “negotiate a mutual slowing down of the SST race”, and that it would be better to provide the financial assistance that Boeing needs by giving the company contracts “to improve our environment rather than destroy it”. He called the SST an “environment despoiling superplane for the jet set”.

The technical case against the SST recited by Senator Proxmire is based on evidence given by Richard L. Garwin, a physicist with IBM who has made a study of the SST project. He argued in May that successive amendments of the contract between the Boeing Company and the Department of Transportation have degraded the performance expected of the SST and that, for example, take-off noise from the aircraft was set at 93 PNdB (perceived noise decibels) in January 1967, but was now expected to be 110 PNdB. With the 125 PNdB of airport noise which might now materialize, the noise pressure at take-off of a supersonic transport could now be expected to be equivalent to the simultaneous departure of 50 jumbo jets. Mr Garwin provided Senator Proxmire with grist for his mill by pointing out that the contract between the US government and the Boeing Company provides no protection for the government against increases of cost, and that there is no assurance that the step from the end of the government's obligations under the contract (in which two prototypes will log 100 hours of flying time) to the commercial operation of the SST will be bridged by private funds. Senator Proxmire's views on the Concorde, chiefly that technical difficulties and inherent defects of design will prevent the aircraft being a commercial threat, are based on evidence to the committee by Miss Mary Goldring, business editor of the *Economist*.

The subcommittee has almost brushed aside the evidence provided in May this year by the two principal witnesses for the Administration, Mr James M. Beggs, Under Secretary of Transportation, and Dr Russell Train, chairman of the Council on Environmental Quality. Mr Beggs was quick to defend the economic prospects of the SST project, saying that the US Government would get its money back if 300 SST machines were sold commercially, and that there are at present reasonable prospects of sales of 600 aircraft. He also claimed that much could yet be done to reduce

the impact of the noise of the SST. Dr Train's evidence might in ordinary circumstances have carried even greater weight, for he asked that “we do not pursue technology for its own sake”, went on to express doubt about the feasibility of reducing the noise produced by the SST to acceptable levels but suggested that the present development programme should be continued up to the point at which a sensible decision could be made. The difference between Dr Train and Senator Proxmire is, of course, that the pressure to save money is now being felt more acutely by legislators than by government officials.

INTERNATIONAL COMMUNICATIONS

Keeping Cables Alive

THE American Telephone and Telegraph Company has made to the Federal Communications Commission what amounts to a plea for some kind of balance between telecommunications satellites and submarine cables. AT&T thus becomes the first of several organizations to respond to the request of the FCC last June for opinions likely to be of use in the working out of long-term policies for international telecommunications.

One of the most striking features of what the company has to say is the sheer scale on which new submarine cables are being planned. Left to itself—and the FCC is required to license all new developments in international telecommunications—AT&T would like to install cables each with a capacity of 3,500 circuits on several Atlantic and Pacific routes by the mid-seventies. The first of these cables would be laid in the Atlantic in the first half of 1976, and would be followed by a second three years later. A similar stretch of cable would be laid between California and Hawaii in 1977. With the new design would go cheap installation—a mere \$8 a circuit mile for the proposed cable compared with more than \$300 a circuit mile for the first fifty-circuit transatlantic cable commissioned in the early fifties and \$30 a circuit mile for more recently installed cables.

Part of AT&T's case for the continued installation of cable circuits is a wish to avoid having all its eggs in one kind of basket. The documents submitted to the FCC point out that in a year and a half to the end of July, AT&T lost more than 400,000 circuit-hours of satellite time but only 100,000 circuit-hours of cable time. AT&T, the largest carrier of overseas telephone traffic from the United States, is at present using roughly 2,000 external circuits, evenly divided between submarine cable and satellites. The company estimates that it will need 8,000 circuits by 1975 and 20,000 by 1980. Although most of the growth in the cable business lies in the transatlantic area, AT&T has ambitious plans for other routes and is, for example, planning to install a 825 circuit cable westwards from Hawaii in the late seventies.

At this stage, the response of the FCC to this view of how to strike a balance between satellites and cables cannot be predicted. It seems probable, however, that the rash of satellite failures that has recently plagued Comsat, together with the difficulties that persist in the negotiation of a new agreement for Intelsat, will ensure that close attention is paid to what AT&T has had to say.

NEWS AND VIEWS

Molecular Basis of Bacterial Sporulation

THE eighteen months have not been wasted which have slipped by since Burgess, Travers, Bautz and Dunn (*Nature*, **221**, 43; 1969) discovered the sigma factor component of DNA dependent RNA polymerase, the enzyme which transcribes messenger RNA off DNA. The concept of sigma factors has been so quickly assimilated that it is hard to credit that in January 1969 it was a complete novelty making headline news. Today sigma factor proteins, which in bacteria play a part in dictating which genes RNA polymerase can transcribe, are central to all attempts to explain the positive control of gene expression. Admittedly, sigma factors have so far only been detected in bacteria and bacteria infected with bacteriophages, where they transiently complex with the core of the RNA polymerase molecules and ensure that transcription of the bacterial or phage genes is initiated at the correct starting points rather than at random. But, in spite of the pitfalls inherent in extrapolations from bacteria to nucleated cells, it has always been tempting to speculate that sigma factors also occur in eukaryotic cells forming part of the complex switch gear required to regulate gene expression during cellular differentiation.

The experiments of Sonensheim, Losick and Shorenstein, reported on pages 906 and 910, certainly make such speculations more plausible. For they have proved that changes in RNA polymerase play an essential role in an example of bacterial differentiation, the sporulation of vegetative *Bacillus subtilis* cells. Bacterial sporulation involves the metamorphosis of a fragile, vegetative bacterial cell into a dormant, resistant spore and an equally striking change in the cell's biochemistry. When *B. subtilis* sporulates, for example, the pattern of enzyme activities and RNA molecules in the cells changes markedly as the vegetative genes are switched off and the sporulation genes are switched on.

But what effects this change over? Sigma factors soon became strong candidates for the job and subsequent experiments strengthened this suggestion by proving that, during phage infection, phage specified sigma factors displace those of the host and subvert the RNA polymerase to the transcription of the phage genes. A new sigma factor, or some other change in RNA polymerase for that matter, made at the onset of sporulation might switch off the vegetative genes and switch on the sporulation genes.

Last year, Losick and Sonensheim (*Nature*, **224**, 35; 1969) found that early in sporulation the template specificity of RNA polymerase in *B. subtilis* changes. The enzyme loses its capacity to transcribe *in vitro* phage DNA and the phage only grows in vegetative cells. Together with Shorenstein they have now taken their analysis of sporulation one step further (page 910) by showing that the polymerase of the vegetative

cells needs a sigma factor of molecular weight 57,000 to transcribe ϕ e DNA but will transcribe poly dAT without the sigma factor. (Two other groups, Kerjan and Szulmajster (*FEBS Lett.*, **5**, 288; 1969) and Avila *et al.* (*Nature*, **226**, 1244; 1970) have also detected this *B. subtilis* sigma factor.) Moreover, Losick *et al.* find that the RNA polymerase from sporulating cells fails to transcribe ϕ e DNA even when the sigma factor is added. Obviously the core enzyme of the sporulating cells differs in function from that of the vegetative cells, for it fails to respond to the vegetative cell sigma factor.

Losick and his colleagues believe they have found the molecular basis of this difference. One of the two β subunits of the core enzyme from sporulating cells has a molecular weight of about 110,000 while both β subunits in the core of the vegetative enzyme have molecular weights of about 155,000. They envisage that at sporulation part of one of the β chains of the core enzyme is cleaved and the enzyme, as a result, loses both its affinity for the vegetative sigma factor and its capacity to transcribe the vegetative genes which are switched off. Losick *et al.* are currently searching for a new sigma factor, which, they suggest, may appear at sporulation, associate with the modified core and specifically direct it to transcribe sporulation genes. In other words, the transcription switch controlling sporulation may well turn out to be cleavage of part of the RNA polymerase core, displacement of the vegetative sigma factor and the appearance of a new sigma factor switching on the spore genes.

The properties of a class of mutant *B. subtilis* isolated by Sonensheim and Losick (see page 906) strongly support this interpretation. The mutants, selected for resistance to rifampicin, which is known to inhibit directly a step in initiation of RNA synthesis, have also lost the capacity to sporulate. The reversion rates of these mutations indicate that they are single events which affect both resistance to rifampicin and sporulation. Furthermore, the RNA polymerase from the mutants retains its capacity to transcribe ϕ e DNA *in vivo* and *in vitro*, even when the cells are grown in conditions which induce the sporulation of wild type cells. Losick and Sonensheim suggest that these single mutations somehow prevent the cleavage of the β subunit of the core RNA polymerase. The mutant enzyme cannot therefore undergo the modification which results in loss of affinity for the vegetative sigma factor and the switching off of the vegetative genes; cells containing the mutant polymerase cannot therefore sporulate. Recently Doi *et al.* (*Proc. US Nat. Acad. Sci.*, **66**, 404; 1970) have also reported the isolation of rifampicin resistant mutants which produce spores of altered morphology. Their work, like that of Losick *et al.*, points to a change in RNA poly-

merase being a critical factor in normal sporulation.

The implication of all these experiments extends, of course, beyond bacterial genetics. Batteries of different sigma factors may well be involved in the coordinated switching on and off of genes during differentiation of eukaryotic cells; even the transformation of cells by tumour viruses can be interpreted in terms of sigma factors. Polyoma and SV40, for example, replicate in some cells but fail to replicate and trans-

form other cells. Perhaps the permissive cells contain sigma factors which allow the complete expression of the viral genome and the replication of the virus, while the sigma factors in non-permissive cells allow only partial expression of the viral genome. In the non-permissive cells the virus would fail to replicate but the viral genome would be available for insertion into the host cell's chromosomes, an event which may lead to the transformation of the host cell.

Advice for Teminizers

TEMINISM—the activity triggered off by Temin's discovery that RNA tumour viruses can reverse the normal direction of flow of genetic information by acting as templates for the synthesis of DNA—is booming. But a major difficulty in assessing progress, which also seems to be confusing many of the research teams now frenetically searching for information reversal enzymes, is how to decide just which enzyme is fulfilling what function. "Know your enzyme" may be a good motto for Teminizers, but it is proving one difficult to live up to.

The one enzyme whose catalytic function is clear is the so-called RNA dependent DNA polymerase (see *Nature*, **226**, 1209 and 1211; 1970). This acts on the viral genome to synthesize DNA complementary to its RNA. So far, however, the extent of RNA-DNA hybrid formation seems to be rather limited; only a small part of each RNA genome is converted into DNA.

The obvious advantage for RNA tumour viruses to be able to convert RNA to DNA is that their genetic information can then be integrated into the genome of the host cell. This would require the synthesis of duplex DNA from the RNA-DNA hybrid, and at least two further enzyme activities are likely to be involved. (This, however, is not to say that the enzymes are necessarily represented by two distinct protein entities; one difficulty of working with viral information-transfer is that as yet the catalytic activities of the virion have not been identified with protein moieties, and one enzyme may well turn out to have more than one catalytic activity.)

If integration of duplex DNA into the cell genome is to be assured, there must first be a replacement of the RNA strand of the hybrid by a DNA strand, which implies one distinct enzyme activity. This single piece of duplex DNA will then probably need replicating so as to provide more copies, which is another enzyme activity. Distinguishing these two enzyme activities *in vitro* is difficult enough, but finding out just how they work *in vivo* may prove impossible until the protein(s) responsible can be characterized. And even these are unlikely to be the only enzyme activities involved. It is possible, for example, that more viral RNA may be synthesized from the DNA-RNA hybrid, and integration of the DNA itself will probably demand yet more enzymes, such as a nuclease to prepare gaps in the cell chromosome at the sites where duplex DNA

is to be inserted. Indeed, the identification of the enzymes involved in this process may well throw light on the vexed issue of recombination, a closely analogous process, the details of which remain largely elusive in more conventional systems.

CANCER VIRUSES

More of the Same

from a Special Correspondent

If people were obliged to publish anonymously, tragicomic situations such as that revealed last week at the 1970 Tumor Virus Meeting at Cold Spring Harbor might be less frequent. If a prize were to be given for the work that has caused the most whip-cracking and unnecessary duplication of experiments, Temin, Mizutani and Baltimore would win hands down. Even the most innocent enthusiasts must now be having second thoughts, however, when so many groups are busily copying each other, characterizing RNA dependent DNA polymerase and the other enzymes in RNA tumour viruses. To judge from the remarks of Dr T. E. O'Connor (National Cancer Institute), however, those who run the institute's Special Virus Cancer Program are all too keen to pour even more money into an instantaneously overcrowded field. He invited the packed hall—the idea that the specialist meeting at Cold Spring Harbor should be intimate and eclectic has gone by the board—to send requests for free samples of RNA tumour viruses to Bethesda as soon as possible. The programme, which must be delighted at the prospect of finding something on which to spend its millions, would then be able to match supply with demand. Feline leukaemia virus is included in the offer, albeit with a mild caution that a virus that jumps species barriers so readily and which grows well in human cells might be dangerous. It is therefore hardly surprising that the standing sick joke has become that, because it is proving hard to find even epidemiological evidence of human RNA cancer viruses, the Special Virus Cancer Program will be content if its pensioners manage to manufacture them in their laboratory.

And what are the fruits of two months' frantic activity? Mizutani and Temin (Wisconsin) have now found two more enzymes in the virions of mouse sarcoma virus—an endonuclease activity which one can speculate may have a role in integrating the viral DNA into the host cell chromosomes on transformation,

and a DNA dependent DNA polymerase which connects the RNA/DNA hybrid made by the RNA-dependent DNA polymerase into double-stranded DNA. Both Spiegelman (Columbia) with his six colleagues and Green (St Louis) with his five colleagues reported that they, too, had detected the enzymes which made double-stranded DNA. Spiegelman also finds that a synthetic polydeoxy-C-polyribo-G is by order of magnitude a better template for the DNA dependent DNA polymerase than anything in the virion. Spiegelman also finds that a polydio-C-polyribo-G is by order of magnitude a better template for DNA-dependent DNA polymerase than anything found in the virion and both Green and Levinson (San Francisco) reported that there are probably small amounts of DNA within the virions of some RNA tumour viruses.

The DNA tumour virologists, who by now must be seriously questioning whether work in DNA tumour viruses will continue to be funded although the discovery of the reverse transcriptase makes the distinction between DNA and RNA tumour viruses little more than academic, held the stage but not the limelight for the first two-thirds of the meeting. Isolation of temperature sensitive mutants of SV40 was reported by Robb and Martin (NIH) and Kit *et al.* (Baylor, Houston) and Eckhart (Salk Institute) described the four classes of polyoma mutants he has isolated, while Hirt and Gesteland (Cold Spring Harbor) and Estes *et al.* (North Carolina) proved that the proteins of SV40 virions closely resemble those of polyoma virions.

PROTEINS

Membranes Dismembered

from our Microbiology Correspondent

By one of those mysterious mass movements, like the swarming of bees or the migration of lemmings, protein chemists seem with one accord to have surged into the membrane field. One's sympathies are with the aboriginal inhabitants, who, after ploughing their lonely and leisurely furrow these many years, have now been overwhelmed by a plague of voracious locusts. Of course there is at this stage no lack of sustenance, for the subject is in a highly interesting state. The newer methods of protein chemistry are starting to pay off, and the systematic attempts now in progress to extract and characterize the proteins of membranes are beginning to impose some sort of pattern.

The attempt to dredge individual components out of the porridge has been motivated either by the desire to localize and isolate a particular biological activity, or by the hope of discovering some features of general structural importance. Both these lines are generously represented in the current literature. One observation of great interest on the proteins of a bacterial membrane has been recorded by Patterson and Lennarz (*Biochem. Biophys. Res. Commun.*, **40**, 408; 1970). Working with an organism presumed to derive from a strain of *B. megatherium*, and dispersing the total protein of the ghosts by treatment with deoxycholate and urea, they fractionate the extract by acrylamide gel electrophoresis and find that no less than 90 per cent of the total protein mass is contained in one component. There is also a series of minor components.

As judged by the detergent-acrylamide gel method, the molecular weight is some 30,000. Examination of the putative parent, *B. megatherium*, strain reveals the usual spread of constituents, one of which corresponds, at least electrophoretically, to the mutant component. On this evidence then, it seems that viable membranes can be assembled from only one major structural protein—a thought-provoking observation.

Phillips and Morrison (*ibid.*, 254), working with red cells, report that when the intact cell is exposed to iodination in conditions involving catalysis by a peroxidase enzyme, only one protein incorporates iodine. It has a molecular weight of about 90,000, and they favour the conclusion that it is the only protein on the outside surface of the cell. It is also possible of course that there are other proteins devoid of accessible tyrosine residues. This sort of experiment will undoubtedly gain currency, however, for exploring the distribution of components in the membrane.

Gwynne and Tanford (*J. Biol. Chem.*, **245**, 3269; 1970) have dispersed red cell stromata with concentrated guanidine hydrochloride, together with reducing and alkylating agents, to eliminate disulphide links. All the proteins were solubilized by this means, and the mixture was then applied to a column of 'Sepharose'. As Tanford and his associates showed earlier, the elution volume of such fully disordered polypeptide chains is a measure of their molecular weight, all the evidence being that only single, unassociated chains can persist in the solvent. At the head of the fractions eluted from the column, Gwynne and Tanford found a giant chain of molecular weight 200,000. This value was confirmed by sedimentation equilibrium. This is in any circumstances a protein of unusual size, and contrasts strangely with the mass of rather low molecular weight proteins previously found to make up the bulk of the red cell membrane.

A more genteel approach to the study of membrane proteins is to try to label and identify active components. Thus Panet and Seliger (*Europ. J. Biochem.*, **14**, 440; 1970) have taken advantage of the stability of the ATPase of sarcoplasmic reticulum, when it is protected by its substrate. In the absence of ATP, treatment of the membrane with thiol reagents leads to the uptake of fourteen labelled reagent molecules per 10^5 g of membrane when detergent is present, or ten when it is not. In either case the ATPase is inactivated. When ATP is present, one of the ten reactive thiols, and also the enzymatic activity, are protected. If all available cysteines are then blocked with an unlabelled alkylating reagent, and after removal of ATP a radioactive alkylating agent is introduced, only the ATPase should take up the label. It turns out indeed that only one radioactive zone appears in gel electrophoresis, compared with eleven alkylated proteins in all, so that the ATPase is unambiguously identified. The same general strategy for isolation of a protein of known function, using instead a labelled specific active site reagent, allowed Bellhorn, Blumenfeld and Gallop (*Biochem. Biophys. Res. Commun.*, **39**, 267; 1970) to purify an acetylcholinesterase from human red cells. Dunham and Hoffman (*Proc. US Nat. Acad. Sci.*, **66**, 936; 1970) have used tritiated ouabain, which is an active-transport inhibitor, to identify its target in the membrane. After solubilizing the total protein, the label is found in a rapidly sedimenting fraction, which coincides, as it should, with the sodium-potassium ATPase activity.

NUCLEIC ACIDS

Rules for Sequencing DNA

from a Correspondent

THE seemingly insurmountable problems involved in sequencing DNA molecules, or even substantial pieces of them, have always provoked stock responses about the necessity of finding specific deoxyribonucleases. This, however, is probably less important than choosing a promising DNA of modest size to work on (such as the reiterated sequences in some mammalian satellite DNAs being studied by Southern and Walker), and using adequate methods for fractionating fragments of it. Some of the technical foundations for the latter task have been laid down recently by K. Murray (*Biochem. J.*, **118**, 831; 1970) in a vast and painstaking study of oligodeoxynucleotide mapping procedures, lasting several years. He started with the methods devised by Sanger and his colleagues which have proved so successful in the sequence analysis of RNAs.

Murray used ^{32}P -labelled DNA prepared from *Escherichia coli* and the bacteriophages λ and fd as substrates. First, he fingerprinted digests of *E. coli* DNA carried out with pancreatic deoxyribonuclease and a deoxyribonuclease from the mould *Neurospora crassa*.

He used the standard method of electrophoresis on a strip of cellulose acetate at pH 3.5, followed by electrophoresis on DEAE-paper at pH 1.9. The fingerprints were qualitatively similar, and reproducible in defined conditions of digestion. Murray then analysed, fully or in part, about seventy spots ranging up to tetranucleotides in size. On the basis of his results, devotees can easily see that the oligodeoxyribonucleotides are fractionated in a similar way to oligoribonucleotides, falling into "graticules" according to their compositions, but the picture is complicated because of the lack of enzyme specificity, so that all the isomers of the smaller sequences arise as products of digestion. Many of these were separated from each other during subsequent fingerprinting, but the very complex mixtures of larger oligonucleotides were not fractionated by this system, and so Murray modified the fingerprinting procedure in two ways. This greatly improved the resolution of these larger products. In one case he carried out the first dimension of electrophoresis at pH 9.7 (5 per cent triethylamine carbonate) on DEAE-paper.

He also used a system in which DEAE-paper was replaced by AE-paper in the second dimension. He indefatigably constructed further maps, after examining about 120 products fractionated on the system using triethylamine carbonate, and another 110 products on fingerprints prepared on AE-paper.

There was, however, a rather unfortunate denouement when Murray attempted to compare the DNAs from *E. coli* and phages λ and fd. Pancreatic deoxyribonuclease fingerprints of these molecules gave essentially similar pictures, and it was not possible to ascribe any definite significance to the slight differences observed. The lesson must surely be that even these comparatively small DNAs are far too complex to be readily amenable to these methods. It will probably be several years before a roll of DEAE-paper and a strong constitution are all that is required to sequence λ DNA.

EARTHQUAKES

Why Chandler Wobble?

from our Geomagnetism Correspondent

ALTHOUGH the Chandler wobble, the precession of the Earth's axis of figure about the axis of rotation, was discovered in 1891, its cause is still a mystery. There are, in fact, two separate puzzles involved—the source of the excitations which produce the wobble of amplitude about 0.5", and the width of the spectral peak which indicates that the period of fourteen months varies within ± 4 per cent. The simplest explanation for the varying period is that it results from continuous excitation of a mechanical system whose natural period changes with time; but it is unlikely that physical changes in the Earth would produce the observed variations over times as short as a year. Alternatively, the Earth could have a fixed Chandler period produced by random excitations which are subject to damping. This is much more reasonable, especially as theoretical calculations of the period turn out to be about 1.20 years.

But what energy source maintains the random oscillations? During recent years it has become popular to imagine that this source is earthquakes; and this view has received considerable support from theoretical calculations which show that, given the right conditions, one large earthquake could produce 10 per cent or more of the observed Chandler wobble amplitude. If a single earthquake can do this, it seems intuitively likely that all earthquakes together could account for the whole of the Chandler wobble. Ben-Menahem and Israel (*Geophys. J.*, **19**, 367; 1970), however, are more pessimistic. They show that a single shallow earthquake of magnitude 8.5, occurring at a suitable latitude and with a favourable strike-azimuth, could maintain the Chandler wobble for about a year—and yet they conclude, paradoxically, that the total number of real earthquakes could account for only about 30 per cent of the observed wobble amplitude and corresponding secular polar shift.

The reason is, of course, that the secular polar shift produced by an earthquake is critically dependent not only on the shock's magnitude but on its position and its strike and source parameters. An earthquake of magnitude 8.5 may maintain the Chandler wobble for a year under optimum conditions; but an earthquake of that magnitude only occurs about once every 4 years, and when it does the conditions for Chandler excitation are far from optimum. If all the annual seismic energy were to be released in a single shallow strike-slip rupture on a meridional fault located at the equator, then again the resulting annual shock would be sufficient to drive the Chandler wobble for ever. In practice, circumstances are far less favourable.

It is important to realize, however, that Ben-Menahem and Israel have derived their conclusions from a mathematical model. It turns out to be a model which is considerably more favourable to the excitation of Chandler wobble by earthquakes than models previously used to describe the same effect—but it is a model none the less, and is therefore subject to simplifying assumptions. Thus Ben-Menahem and Israel have not proved conclusively that earthquakes do not account for Chandler wobble because some assumptions may not be valid and modifying conditions may remain to be discovered. For this reason, we have certainly not heard the last of earthquakes as the source of Chandler wobble.

New Knowledge for Old

by

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This article is a shortened version of the fourth annual Aslib lecture, which Professor Ziman delivered on April 22 under the title "The Light of Knowledge: New Lamps for Old".

At last year's meeting of the British Association¹, I tried to give an account of the communication system of science, and to explain its rationale as a social organization. So much has been preached and prophesied against our conventional system that it seemed useful to make explicit the function that it is designed to serve and the reasons for some of its peculiar features, before we decide to take it all to pieces and build a far far better world.

This time, I want to ask a different question: what sort of communication system will the scientist of the future devise, instinctively, to suit his needs? Since we know nothing for sure about this hypothetical person, this article is pure speculation; but science fiction may be as effective as history in the spinning of fables and the manufacture of myths.

The Conventional System

First I shall draw attention to three characteristics of the orthodox system of scientific communication, as we may find it in learned journals, treatises, and handbooks. The primary function of an ordinary scientific paper is to bring into the public domain an explicit account of some new scientific development or discovery. As I have argued at length², the whole activity of science is dependent on the publication of such documents, and their subsequent criticism, re-evaluation, and eventual acceptance or rejection as parts of the consensus of "Public Knowledge".

But scientific publication is also essential in the social system of science as the registration of priority of discovery. In other words, since research is the production of new, useful, "consensible" knowledge, the original papers of a research worker are the tangible evidence of his ability to do research.

Finally, I would draw attention to a uniform characteristic of the whole conventional system: the user of the information pays all the costs of publication. Books and journals are published by learned societies and commercial organizations, and sold for profit—or at least to cover all the printing costs—to individual research workers or to the organizations for which they work. The library is a cooperative of readers, not authors. This is a point of some principle, for it is the ultimate source of the power of the scientific editor or referee or librarian; he may reject a work on the grounds that it will lower the standard of his journal, or monograph series, and hence endanger its financial viability.

Crisis of Over-population

This conventional system is now in a state of crisis, due chiefly to the enormous expansion of scientific activity in the past few decades.

I shall look at this crisis from three aspects. Much discussion has concentrated on the difficulties of indexing, abstracting, and assimilating the vast quantity of new

material, proliferating from many breeding grounds and overrunning the poor scientist who is just trying to keep up with the literature. This seems to me to be largely a technical problem that can be solved quite effectively if we are prepared to put sufficient resources into it. But a "technological fix" with arrays of half-witted computers is not the only answer: there is still plenty of scope for development in the old fashioned procedures of compiling, indexing and printing up-to-date collections of abstracts and reviews.

A more intractable consequence of the expansion of science is the debasement of the criteria of publication. Not only is there too much scientific work being published; there is much too much of it. The need to get recognition by publication forces each of us to shout a little longer and louder so as to be noticed at all in the swelling crowd of voices. If we cannot get into the quality journals, we send our pitiful contributions to a second rate publication, produced perhaps by a commercial publisher who has learned that vanity will get him a distinguished-looking list of editors and the professional zeal of librarians will ensure that he gets his money back. Or we may simply have our work printed, or duplicated, as a "technical report" or (hopefully) as a "preprint", and send it round free to everybody whom we think ought to be interested. However hard we try as editors to keep up standards, the referee system is being eroded at the edges.

Another serious consequence of the expansion of science is a steep rise in the cost of information services. If we conceive of an information system as a network of pathways from individual to individual, then N individuals generate $\frac{1}{2}N(N-1)$ separate channels of communication. Thus, as N increases, the number of channels required by each individual also increases. If the bulk of literature in his subject is growing, then it costs him more to purchase all the journals and books he needs, whether from his private purse or through an institutional library. For the individual scientist these costs can be kept down by only purchasing material within his own specific field. But for institutions such as universities, this economy is not available, and scientific library costs are really beginning to hurt. In rich countries, this extra burden can be carried; but for many developing countries, and especially those states with pathological objections to the expenditure of foreign exchange, scientific progress is now hobbled almost to a standstill by the inability to buy the full range of publications that now contain the modern stock of scientific knowledge. The attempt to solve the information crisis by computerized retrieval aggravates the position of a university or research institute in India, Africa, or Latin America, for whom these gadgets are much too expensive. I do not know whether anyone has calculated the cost of such devices per research worker using them, but it would be tragic if they came to seem indispensable.

Industrialization of Science

It is my impression, however, that discussion of the information crisis has seldom been accompanied by a recognition of the rapid structural changes that are taking place within the scientific community which the communication network is supposed to serve. The tacit assumption of the librarian, journal editor, information officer or scientific publisher is that he will always be dealing with the products and needs of individuals.

It is obvious, however, that scientific research is rapidly evolving from a cottage industry to a system of factory production. This, surely, is an inevitable consequence (if not, also, a cause) of the recent enormous expansion of science. Is this not the appropriate simile for those vast hives of scientific buzzing and honeymaking that we now observe throughout the world, those national laboratories, research institutions, institutes for atomic energy, or for cancer research, or for apple growing, or for seaweed, or for noise, or for space, or for higher thoughts, or for lower temperatures, or for advanced studies, or for retarded minds? Faraday and Edison, Pasteur and Darwin—they were individual artists or artisans, with perhaps a few apprentices as extra hands; their successors are executives, captains of industry, directors of research, managers of departments, chairmen of research councils, and other high-powered bureaucrats.

To some extent this development flows from the demands of "big science"—especially in applied research and development. There are economies of scale and natural psychological tendencies; not to mention factors of efficiency and tidiness in the minds of the administrators of government grants. Whatever the reasons, good or bad, the fact of industrialization and bureaucratization of science remains.

The consequences of this historical development for the life of the mind have not been discussed seriously; perhaps we cannot discern the effects ourselves, as the wave breaks over our heads. But I would draw attention to two obvious changes in the personal status of the scientist: he becomes more professional, and more subordinate to a team activity.

Until quite recently, the bulk of scientific research was done by university teachers, not as an explicit part of their employment but as a semi-independent, personally directed activity, motivated by the desire for further preferment as a reward for scholarly prowess. This pressure could be agonizing⁴—witness the bitter struggles of Sigmund Freud to achieve recognition for his discoveries—but it was indirect and unspecific. The official doctrine was that a university lecturer, being called to the vocation of high scholarship, would naturally wish to add to the corpus of pure learning, and was given the freedom to make the best of his time in that way. Within the bureaucratic institutions of our day, this amateur status has been renounced. Whatever freedom he may have in the day-to-day, month-to-month, even year-to-year direction of his research, the modern scientist is none the less paid to be a research worker, and is expected to produce some results of a publishable or useful kind, in return for his stipend.

In the best research institutions, an effort is made to preserve the freedom of the individual scientist in his choice of problem and in the tactics of his research. Nevertheless, the tendency towards team work grows ever stronger. In big science⁵, the experiment, and the discovery that arises from it, is not a product of the ingenuity of a single mind, but of a large group, with members who specialize on different aspects. The evidence for this

multiple effort is the multiple authorship of the paper in which the results are reported—a dozen, a score, even half a hundred names appended to the publication. The individual skills required may be of the highest order, but the "productivity" or "creativity" of each participant is merged into the collective mind of the team. An investigation that costs a million dollars is clearly a large scale social activity, not at all the same thing as a personal work of art for which credit may be assigned to the individual creator. The very idea of tussling with a problem of natural philosophy may be forgotten in the struggle to achieve some immediate technical aim within the vast framework of the apparatus.

Effects of Industrialization

What will be the effect of this change in the social and psychological structure of science on the information system? First, it is obvious that there is a grave threat to the convention of awarding promotion, or other forms of recognition, on the strength of published work. The mere fact that a candidate for a lectureship in elementary particle physics has his name among the dozens of "authors" of some significant discovery says little about his scientific skill. In the long run, the leader of such a team gets the credit for its contributions to knowledge, but he must be already the selected and tested boss of a big group. Evidence of ability at a more junior level can only be assessed within the framework of the project itself, just as it would be in an army, a civil service or other bureaucracy. With direct power in the hands of the seniors, one of the primary functions of the conventional communication system of science is losing weight. The necessity of maintaining an open market for the creations of the individual scholar, as objective evidence of achievement and promise, is no longer evident.

On the other hand, the funding of research on a vast scale, the assumption that every scientist is a professional, employed and supported materially by a large scale institution, makes it easier to find the money for expensive new techniques of communication. We begin to take it for granted, for example, that we may charge to laboratory overheads our long-distance telephone calls, which we would never dream of paying ourselves. Air mail for reprints, air travel for conference participants, secretarial assistance, Xerox machines, electric typewriters, photo-offset printing, all are enlisted in the battle for more and more rapid, total, instant, informal communication. It is easy to prove that such aids, although not of negligible absolute cost, pay for themselves many times over within the context of million dollar research budgets, palatial buildings, gargantuan machinery and armies of assistants.

The question that has not been resolved is how this money is best spent in the support of the formal communication system. In practice there are two divergent trends. On the one hand, the provision of more and more lavish library facilities is taken for granted, along with the new gadgets for information retrieval and search. As I have remarked, the affluence of science in the more industrialized nations can easily keep pace with the flood of new publications. The assumption is made that library funds will always be increased to permit the purchase of all the new periodicals and books that are relevant to the research in hand, with lavish allowances for treasures that may still be unearthed in the older literature. The recognition that these costs are really trivial by comparison with overall expenditure on buildings, equipment, technical staff and professional salaries was the secret of

at least one great fortune in the technical publishing world—and it remains largely true today. Some scientists have jibbed at the enormous expense of such marginal aids as the *Science Citation Index*, and are worried at the high prices now being charged for advanced treatises and other books, but they can still maintain an open-ended commitment to obtain all the genuine literature of science without making deep inroads into overall research budgets. Thus, the financial power of bureaucratic science is put at the disposal of the users of information; the larger the scale of the laboratory, the easier it is to provide such facilities on a collective basis.

On the other hand, this financial power is being applied to the information system at the producer end. I have already referred to the growth of the "Report" literature, especially within the very large government agencies and industrial laboratories. This material may not be produced in the full glory of letterpress, but the re-typing and duplicating of a few hundred copies of a 20 page report or preprint, not to mention air-mail postage and incidental overheads, is not a negligible expense. The fact that it is distributed free—that it is almost forced upon one, like an advertisement for laboratory equipment or life insurance—is in striking contrast to the high subscription one has to pay to read the same article, only a few months later, in a regular journal. The pressure to get into print, for professional prestige and preferment, is thus diverting a substantial amount of research funds into the information system, but in an extravagant manner that is largely dysfunctional to the scientific community, through the degradation of standards and the clogging of the channels by multiple publication of the same basic research.

A parallel development is the rapid growth of the page-charge system. The ancient custom of supplying offprints is not begrudged as a service to friends and colleagues who might find it convenient to have a good printed version of the article ready to hand. But this has now been inflated into a really steep charge—\$80 per page in the *Physical Review*—levied on the institution of the author when the paper is accepted for publication. It is argued that publication is part of the overall research activity, and therefore its cost should be met out of the research grant, or other fund, that pays the salaries and equipment of the researchers.

This is perfectly logical: but it does conflict with the general principle that publication of scientific information is paid for—hence, eventually controlled in quality—by the reader. The actual pressure behind page charges is the desire of each editor to keep the selling price of his journal within the means of the individual subscriber. This is particularly so for the great scientific societies, whose members have become accustomed to receiving their journals at quite a modest price, as a special concession and a service. I belong to the American Physical Society, not out of international brotherhood and solidarity, but because I can get a great deal of valuable scientific material—several thousand pages a year—for a subscription of only \$50 or so. It is a good bargain, and would be particularly significant if I were working in a developing country where library costs were a real burden.

Nevertheless, page charges become a tax on research, and can only be borne by heavily bureaucratized science, where the employee can assume that somebody else is picking up all the checks. The small research group, still trying to produce in the cottage industry mode, gets squeezed out. Is it really the intention of the American

Institute of Physics to prohibit in its journals any publications from the outside, dollar-less world? I certainly do not propose to spend the salary of a good research assistant on having the papers from my own research group published in the *Physical Review*, when I get them printed for free in the corresponding British journal. The AIP says, of course, that the charge is not obligatory: but it is now delaying publication of papers from "irresponsible" institutions that do not "honour" their page charges, and has begun to ask higher subscriptions from foreign members on the grounds that we are not paying our fair share of the printing costs. In other words, we are approaching a situation in which the whole scientific community of the United States is regarded as a single body, supported financially and thereby controlled by the major funding agencies of the government. This support is not a disinterested gift to learning and to humanity, but it is evidently calculated as the cost of buying certain specific services for the nation.

I do not propose to discover conspiracies, nor to denounce the more sinister aspects of the world we live in: in any case, the brutal bureaucratization of science in the Soviet Union and its colonies is far more tragic and foolish than any situation they can manage to bungle their way into, through sheer expediency, in the United States. The significance for the information system is that the institutions that employ the scientists and treat them as a category of skilled manpower are now laying their hands on the information system, and beginning to take charge of the mechanisms for the publication of knowledge.

We are now in a state of transition. The individual scientist—or at least the leader of the research group—is allowed to say what he likes, how he likes, and takes personal responsibility for his research results just as if he were an old-fashioned professor. He may, indeed, have at his disposal the funds to advertise his work through the distribution of reports and preprints, without any critical check. On the other hand, he must still submit his results to the scrutiny of independent anonymous referees if he wishes to see them published in a respectable journal. The financial power of the laboratory that employs him is not used to interfere significantly in the form and substance of his published work.

How long can this situation continue? Will there be, before long, a tightening of the strings within the bureaucratic organization, so that the decision to support publication will rest with higher authorities than the man who really did the research? In a new historical phase where money has suddenly become tighter for research, it seems inevitable that more careful control of page-charge, report, and reprint costs will be exercised, so that only work judged worthy by the senior officers within the institution will be cleared for publication. To anyone familiar with scientific controversy, and the self-righteous malice of the second rate senior scholar found in the wrong, this is an unhappy prospect. The history of Lysenkoism⁶ shows the destructive effect of ignorance and incompetence when it gets its hands on the levers of power of a complete bureaucracy, and controls publications as well as employment. We could see a covert conspiracy of the publishing monopolies with the scientific institutions, in which awkwardly critical or uncomfortably heterodox research could get no public hearing.

I am not quite so pessimistic as this. I suspect that the institutions will take firmer control of the publications of their employees, and insist on higher standards of style and achievement before they will pay the bills. In

other words, the published work emanating from a particular laboratory may be seen more and more as a product of that laboratory, rather than as the creation of an individual scientist who only works there (witness, for example, a paper by "Orsay Liquid Crystal Group" in ref. 7). As it becomes more difficult to make a name for oneself by one's personal list of publications, the pressure to put on the market vast quantities of half-baked material may recede, and a longer rhythm of work, with less frequent, less fragmentary papers, may succeed. Of course, there will still be tremendous competition for priority of discovery between groups in different laboratories, but this may well be catered for through the informal system of letters, preprints, and so on. The actual number of papers ceases to be of much significance as a standard of productivity by a substantial institution.

We could arrive at a situation where in practice the role of the referee, as a professional critic, would be performed mainly within the producing laboratories, or even within the research team itself. It must be remembered that the function of the editor of a journal, or of his anonymous referees, is not to guarantee the complete scientific validity of every paper that eventually gets published. One only finds this notion in the minds of certain notoriously hypercritical scholars, who eventually get dropped from the panel of experts! The job of the referee is to confirm that the work is scientifically interesting, more or less original, reasonably well expressed, and not vitiated by obvious errors that the author can be persuaded to acknowledge. It provides a barrier against irrelevance, crankiness and gross incompetence—not against mild stupidity or subtle mistakes. These conditions can be met quite easily by the informed critical examination of work in progress within a capable team or well-established laboratory. In other words, I am prepared to believe that the standard of work published at the behest, and in the name, of a well-established laboratory or other scientific organization might be somewhat higher than the average produced by individuals in the current literature.

Shall we then see a new system, in which the great scientific corporations announce their discoveries in "house journals", edited internally and distributed free? In general, such a system would not conflict with the fundamental characterization of science as a community devoted to the creation of "consensible" knowledge. Given reasonable freedom of speech and action within the corporate institutions, critical comment and the eventual arrival at a rational consensus would still be achieved. The "scientific attitude" has become so engrained in the thinking of a generation of professional research workers that even such power structures as Lysenkoism can be defeated by an appeal to simple fact and straight logic⁶.

But it seems essential for the preservation of this attitude that the bureaucratic corporations should not monopolize the sources of information and means of communication. One single international institute of high energy physics, dominated by its trillion volt machine and controlling all the media of publication in this field, would be a monstrosity. Competition for priority between individuals must be transformed into intellectual controversy between co-equal groups and teams, with public debate and open criticism the major weapons.

It is particularly important that the old style journals, published at the expense of their readers and preserving high standards through the use of anonymous referees, should not fall for the expedient device of the page charge.

As I have said, there is a danger that the learned societies may fall in with the bureaucratic trend, and fail to protect the rights of those of their members who still have freedom to do research on their own account. Can't you just see it? A paper arrives from a private address. "Don't know this name. . . . Doesn't seem to be in the directory of PhDs. . . . Where does he work? Seems to be doing some desk job in the Patent Office. . . . They don't have a programme in astronomy, surely. Has he got permission to publish from his research manager? I bet they won't pay their thousand quid for this stuff. I'll call up the Director: what was the initial; oh yes: A for Albert—OK, I've got it. Albert Einstein."

To maintain the older system of scientific communication within the framework of the new bureaucracy and the new technology will not be cheap; to many, it may seem no more than the preservation of a charming traditional custom. The new procedures which I have sketched out will have many advantages, permitting extensive rationalization of the information network, and much easier access to libraries, computerized indexes and other means of information retrieval. Whatever is published by the great laboratories and other corporate bodies may be better written, better printed, more uniformly of scientific interest, and better indexed than anything in the past. For normal science—the solving of puzzles within an accepted framework of ideas, by well organized teams, using expensive apparatus, under the direction of experienced professionals—this may be the best way to do it. But the mechanisms for revolutionary transformations of thought such as those generated by a Darwin, a Mendel, a Wegener, a Planck, have always been frail, and must be preserved at all costs. I am sorry for the Indonesians and Bolivians, unable to buy all the journals they need to keep themselves scientifically informed, but I do not think it would be to their benefit if the world's scientific literature were given away, like advertisements for patent medicines, yet all doors were then closed to the publication of their own modest researches.

Books and Bureaucrats

I have left to the last the very important problem of reviews and books within the framework of big, corporate science. As I have already suggested, the supposed crisis in technical book publishing, with hard cover or even paperback editions pricing themselves out of the market⁸, is not the real issue: the difficulty is to get them written in the first place. There is already a serious lack of incentive towards review writing, instead of primary research¹, fostered by a false philosophy of originality and "creativity". It is held to be a waste of talents (however modest!) to "take time off from research" for the supposed drudgery of this "derivative activity". Even within the conventional system, the writing of books and review articles has become intellectually devalued, and is no longer regarded as a high art of scholarship, contributing greatly to the furtherance of knowledge and winning laurels of fame for the author.

Of course, the financial rewards are usually modest. If scientific books were composed by professional writers, then we should have a depressed class of hacks on our hands, as poor as would-be novelists and infinitely duller. In practice most reviews and books are written by people already enjoying a full academic or other institutional income.

The bureaucratization of science weights the balance even more heavily against the writing of reviews and

books. It is psychologically much more difficult for the member of a team to withdraw himself from active experimentation than it has been, in the past, for the lone individual. Imagine yourself, once more, in a group devoted to high energy physics or to space research. The experiment has been planned several years previously, as part of a complex continuing programme. The technique of observation, the design of the apparatus, the testing, proving, and running of the equipment, are all novel, and only marginally reliable. The whole process, spread over many months, is a race against time. There are continual crises, and conferences, and unplanned modifications to develop and test: and skilled manpower is in short supply. How can one possibly withdraw from such activity with its clear and immediate goals just to sit, and think, and write a few hundred words, and scratch it out again, and wander aimlessly round the library seeking inspiration, and go back to the desk, and try again to express with due clarity and simplicity the convoluted thought one has nearly mastered in another man's work. In the busy workaday world of making, mending and doing, this looks like *dolce far niente*, or even treason: "We've no room for passengers on this craft," says the leader of the team—handing one a monkey wrench, and indicating the vacant place around the apparatus where it is to be applied. If one is trained from manhood to work in such a team, one can scarcely abandon one's friends and colleagues in such circumstances.

Even the individual, lone scientist, employed by a university or other institution, may come to feel that he is not really earning his salary if he spends his working time in this apparently unproductive activity. As can readily be shown, by an appeal to the general principle that science is public knowledge, necessarily subject to a continuing process of critical assessment and re-integration, the activity of the review writer is quite as important to the scientific community as the making of "original" discoveries—but the purpose of the bureaucratic research corporation does not extend that far. We face, indeed, one of the familiar paradoxes of society—the "crisis of the commons"⁸—in which the immediate self-interest of the individual is best served by his adding another goat to the grazing herd, rather than by fencing and controlling the use of the common land. In so far as the individual becomes the paid employee of the purely selfish and soulless corporation, he loses his liberty to act in an enlightened way in recognition of the needs of the community. In other words, the shallow philosophy of "getting on with one's work"—that is, piling up research findings—is actively reinforced by the attitudes of management, dominated by quantitative measures of "productivity" and "creativity" and not caring at all about the general health of the art.

Review and book writing is thus driven into the "spare time" of the scholar—evenings, week-ends, and occasional sabbatical leaves. Even in the university it is not considered proper for any scientist except a professional "theoretician" to spend his ordinary working hours in this way. There could develop a threat to the modest financial rewards to be won by this extra effort—high taxation on additional earnings, or the demand that "outside" income by holders of academic posts be disclosed and curtailed in the name of equality, fair shares, and maximum productivity.

We must evidently begin a campaign now, before some sort of disaster of fragmentation falls upon the whole system of communication in science. A number of lines

of action suggest themselves. The providers of research grants, for example, might consider much more seriously the explicit subsidization of such writing, by providing funds for sabbatical leave that would free a university teacher from his everyday duties for a certain time, not to do more experiments but specifically to write. There have been occasions when the writing of a book has been proposed as the goal of a research project, but from what one hears this is not usually received with favour by the expert advisory committees, who perhaps set much higher standards for what they regard as the essentially mechanical work of surveying what is already known than they do in estimating the likelihood of success of a proposed new investigation. They must be made aware of their own unconscious belief that writing is really much more difficult and dangerous than experimentation (for it exposes the mind and soul to criticism and ridicule) and therefore be lenient and generous towards those who are willing to give it a try.

Within "big science", it may be necessary to assign the role of "scribe" or "theorist at large" to a particular member of the team, or to provide income and facilities for a number of such experts within the institutional framework. Perhaps this is already happening—for example in the enormous space-science corporations and administrations. But notice the danger of specializing and professionalizing this particular role. The knowledge and learning to write a review or book come only by direct experience at the research front, by participation in the uncertainty of experiment and argumentation: no amount of external reading around a subject can give one the feel of it. Thus, every research scientist ought, from time to time, to put on for himself the mask of the reviewer, to see his subject whole and express his own wisdom; he cannot leave this essential task to a hired writer, however expert at grammar and syntax. The invitation to assume this persona could as properly come from his research manager or other bureaucratic superior as any instructions concerning research to be undertaken or classes to be taught.

Finally, the new tendency for the cost of the information system to be met by the producer rather than the reader might be allowed to take effect here too. The books written by those employed for that purpose by corporate bodies could be produced cheaply, and heavily subsidized, in the name of scientific progress. If the incentive of royalties and other personal rewards is still to be active, then a substantial subsidy to non-profit publishers might make it much more worthwhile for authors to stop research or teaching for a while. The book-writing world, fiction and non-fiction, has such a peculiar economic structure, and seems to defy so many of the theorems of the market place and factory, that one cannot predict just where and how the extra funds would have the best leverage—but it seems to me essential that corporate action be taken to restore to the scientific community as a whole these services that are now threatened.

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Unification of Foetal and Neonatal Immunology

by

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By applying age equivalence to available information it is possible to show that the onset of immunocompetence occurs at the same stage of physiological development in all mammals and birds.

RAPIDLY increasing knowledge of immunological function in the fetuses of many animals emphasizes the need for some means of comparison between the species. Animals pass through similar morphological and physiological phases during foetal development, yet some species seem to become immunocompetent long before others. If some method of direct comparison of physiological age could be devised, we might pinpoint the significant stages in the immunological development of all animals. In this article I assemble and compare the present somewhat fragmentary knowledge of the onset of immunocompetence among various species by age equivalence, and confirm that animals become immunocompetent at a similar stage of physiological development.

Sirotonin¹ and Zdrodowski² have proposed that the immunological maturity of a newborn animal could be related to its physiological maturity. The chief difficulty is to define "physiological maturity"—a question I have discussed at length³. Witschi⁴ made a pertinent contribution in emphasizing that birth occurs at different ages not only in chronological time but in terms of the degree of physiological development. Clearly, a hamster, born after only 16 days of gestation, is much less mature than a newborn guinea-pig which has been *in utero* for no less than 62–65 days. The student of foetal immunology would like to know if a newborn guinea-pig is immunologically as mature as a rabbit at birth and if any direct comparisons of immunological status can be made with a newborn human baby. I shall attempt to offer an answer to such problems.

Asdell⁵ and Witschi⁴ showed quite clearly that small animals (with short gestational periods) take a remarkably uniform time (6.5 days) to reach the primitive streak stage in embryonic development, while larger animals (with longer gestational periods) require 11–19 days. The opossum is born only 6 days after reaching the primitive streak stage, and the hamster after only 9–10 days, so it should not be surprising to find that these are mammals immunologically immature at birth. Asdell⁵ extended his idea to later stages of embryonic development and demonstrated that mammals take a similar percentage time to reach a given morphological stage of early foetal development.

Lymphocytes and Growth Curves

Otis and Brent⁶ compared morphologically equivalent stages of mouse and human embryos and plotted them in gestational times. Using their figure a direct comparison can be made between the first appearance of small lymphocytes in mice and men. These cells have been observed at 7–8 weeks of gestation in the human and between 14–15 days in the foetal mouse³. Such periods of development coincide on the graph and show that lymphocytopoiesis occurs at the same equivalent embryonic age in two species of quite different size, life span and length of gestation. Unfortunately, we lack comparative embryological data for all the species with which we are concerned. This particularly applies to later stages of foetal development. I do not plan, at this stage, to

embark on a comparative morphological survey to obtain age equivalence based on embryonic differentiation: such a method is certainly desirable, but it unfortunately depends on many embryological observations instead of a parameter which is more readily measured, such as body weight.

If the growth curves of foetal body weight of each species could be unified in a common curve, the times of age equivalence for all species could be read from any point on this curve. Brody's classical work⁷ on common post-natal growth curves has been applied to foetal life by Weinbach⁸, who modified Brody's formula

$$W/A = 1 - e^{-k(t-t')}$$

where W is the weight of the foetus at time t , and t' is the age at which the extrapolated curve cuts the age axis (a mathematical approximation to the onset of embryogenesis). In Brody's formula, A was taken as the weight of the mature animal, but in Weinbach's method A is calculated from three equidistant points (w_1, t_1) , (w_2, t_2) and (w_3, t_3) on the foetal body weight/time growth curve using

$$A = \frac{w^2 - w_1 w_3}{w_1 + w_3 - 2w_2}$$

The other constants are calculated from

$$k = \frac{2 \log_e \left(\frac{w_2 - w_1}{w_3 - w_2} \right)}{t_3 - t_1}$$

$$t' = t_1 - \frac{1}{k} \log_e \left(\frac{A + W}{A} \right)$$

From the three constants A , k and t' and the body weights w_1 , w_2 and w_3 , W/A is plotted against $k(t-t')$ for each species. The three values of t in the developmental age range of each animal are obtained from the three corresponding values, on the horizontal axis, of $k(t-t')$.

My first application of this formula to foetal growth—for all species which have been studied immunologically—indicated that although there was good agreement between the equivalent times of onset of immunocompetence between animals with gestational periods of more than 30 days, this group differed in $k(t-t')$ values from animals with short periods of gestation. There was also good agreement with respect to the onset of immunocompetence among members of this second group of animals. For the method to embrace all species of animals a modification had to be made to include those mammals and birds with relatively short gestational periods. Through Zdrodowski's² proposition "that physiological maturity is a necessary criterion for immunological maturity; the fact that an animal is born does not in itself imply that it is immunologically mature", I was led to Tanner's work on growth velocity. Tanner⁹ emphasized that in species such as man and guinea-pigs, with relatively long periods of gestation, there is an increasing gain in weight ("weight velocity") until birth, when there is a change to slower body growth. These species have the highest birth weight relative to their mother's weight. Other species

such as the rabbit, rat, mouse, hamster and chicken are born "too early" and their weight continues to increase during post-natal life. The rabbit is an extreme example—it experiences an overall increase from 20 days of gestation until 40 days after birth¹⁰. The rabbit thus achieves its peak increase in weight at a very similar time (after conception) to the guinea-pig. The daily increase in weight of rats and mice also continues to accelerate after birth; peak growth rate may coincide with the time of weaning. Tanner's work suggests that animals with gestation periods up to 30 days are born before they have had sufficient time to reach a certain degree of physiological maturity, which is not attained until some time after birth. I have taken the end-point, t_3 , of the common growth curve as the time of "peak growth velocity" for all species. For simplicity, I have assumed that animals with periods of gestation greater than 60 days have their "peak growth velocity" at birth. This is based on the substantial evidence assembled by Tanner⁹. A further improvement to Weinbach's formula is to take t_1 as the time of first appearance of small lymphocytes in the thymus. On this basis, the growth curve covers the period of development from the onset of lymphocytopoiesis to the time of peak growth velocity.

Growth constants have been calculated on these criteria³. Data were obtained from sight-fitted growth curves plotted from foetal weights for the chicken¹¹, hamster¹², mouse¹³, rat¹⁴, rabbit¹⁵, opossum¹⁶, guinea-pig¹⁷, cat¹⁸, dog¹⁹, pig²⁰, sheep²¹, cattle²², monkey²³ and man²⁴. Post-natal growth data were obtained from publications about the hamster²⁵, albino mouse²⁶, White Leghorn chicken²⁷, Long-Evans rat²⁷ and rabbit²⁷.

Data for the time of first appearance of small lymphocytes (t_1) in different species, obtained from ref. 3, are given as the figures on the extreme left of the age ranges in Figs. 1 and 2. The time of "peak growth velocity" for animals with short gestational periods was calculated from post-natal growth data and this is expressed at the extreme right of each age scale in Fig. 2. The times of onset of transplantation immunity, γ M-globulin and antibody formation are indicated on each age scale when the information is available.

The value of $k(t-t')$ for t_1 is usually very close to zero (except for the dog). The first appearance of small lymphocytes in the thymus is the beginning of all foetal immunology based on the lymphoreticular system. The age when this occurs is not easy to define accurately, particularly in larger animals, and further study on lymphocytopoiesis may help to remove any discrepancies. The dog seems to warrant further investigation, for the earliest reported lymphocyte in this species appears long before that in the cat, which has a very similar period of gestation.

The times of onset of transplantation immunity and initial reactivity to phytohaemagglutinin are given in Table 1. Transplantation immunity is not easy to measure: it may be defined as the first influx of proliferating small lymphocytes in a graft bed or the time of final rejection of a skin graft. To avoid confusion I have taken the age when allografts are not usually accepted for longer than 15 days as a definition of immunocompetence. This would correspond, in humoral antibody formation, to the early inductive period. A better criterion would be the onset of the lymphoproliferative reaction (usually about 6 days after grafting), but sufficient data are not yet available. Thus the equivalent ages in Table 1 are not directly comparable with time of onset of antibody formation. Examination of $k(t-t')$ values (Table 1) reveals that the

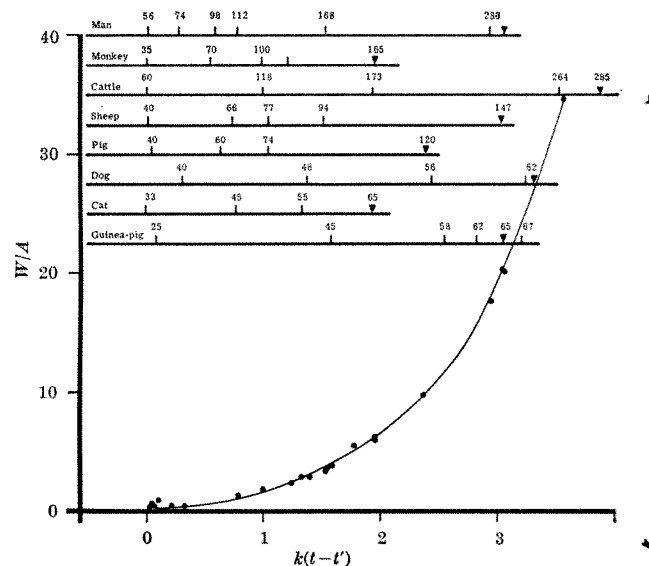


Fig. 1. Age equivalence of foetal primates, artiodactyla, carnivores and guinea-pig. Birth is indicated by ∇ . The figures at each end of the age scales refer to the first appearance of small lymphocytes (t_1) and the approximate time of "peak growth velocity" (t_3). Intermediate figures are the mid-point (t_2) and the times of onset of transplantation immunity, γ M-globulin and antibody production (from data in Tables 1, 2 and 3).

opossum, rabbit, rat, mouse and chicken, and possibly the dog, become immunocompetent at much the same physiological age ($k(t-t') = 0.18-0.43$) and considerably earlier than the sheep (1.04), which is the only large animal to have been skin grafted *in utero*. Data on man and monkey suggest that cellular immunity and resistance to attack by allogeneic cells, respectively, are established at a similar physiological age ($k(t-t') = 0.55-0.60$) as the ability to reject a skin graft in smaller animals. Calves and guinea-pigs have not yet been studied in early foetal life; the need for such an investigation is indicated by their remarkably high $k(t-t')$ values. There is good evidence that foetal guinea-pigs become resistant to viral attack from about 40 days of age³, and from Fig. 1 we can predict that they may become immunocompetent by 30 days of gestation.

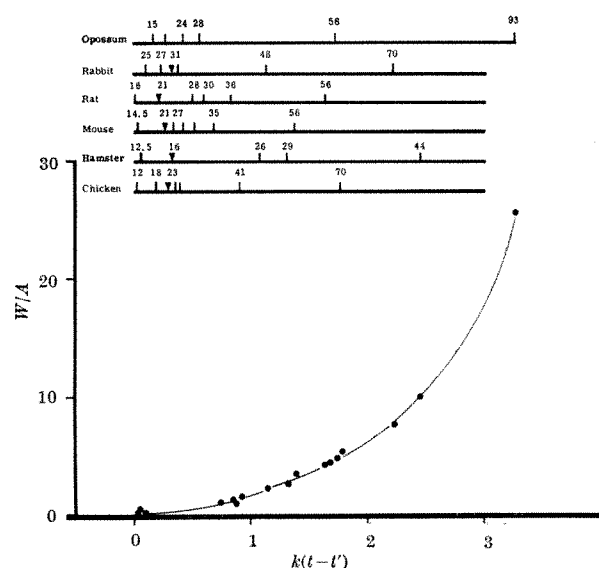


Fig. 2. Age equivalence of foetal and post-natal rodents and chicken. Birth or hatching is indicated by ∇ . The figures at each end of the age scale refer to the first appearance of small lymphocytes (t_1) and the approximate time of "peak growth velocity" (t_3). Intermediate figures are the mid-point (t_2) and the times of onset of transplantation immunity, γ M-globulin and antibody production (from data in Tables 1, 2 and 3).

Table 1. ONSET OF TRANSPLANTATION IMMUNITY OR DELAYED HYPERSENSITIVITY

Species	Onset of transplantation immunity* (days post coitus)	Age equivalent $k(t-t')$	References
Man	98†	0.60	29
Monkey	70‡	0.55	30
Cattle	264	3.52	31
Sheep	77	1.04	32
Dog	40-48§	0.33-1.38	33
Opossum	24	0.43	34
Guinea-pig	62	2.83	35
Rabbit	27	0.18	35
Rat	21	0.22	36
Mouse	21	0.30	37
Chicken	23	0.36	38

* Age when allografts are not usually accepted for longer than 15 days.

† Proliferative reaction to phytohaemagglutinin.

‡ Resistance to graft versus host attack.

§ Delayed rejection.

Fewer species have so far been studied for their ability to synthesize γ M-globulin (Table 2). Man and chicken are able to synthesize γ M-globulins at a remarkably early age: $k(t-t') = 0.20-0.29$. These figures suggest that other species, such as the pig and rat, may soon be found to produce γ M-globulins rather earlier in development, particularly if more sensitive methods can be used. The guinea-pig is clearly an appropriate candidate for study—from Table 2 and Fig. 1 we can predict that it might be capable of γ M-globulin synthesis at a time as early as 30 days of gestation.

Table 2. THE EARLIEST APPEARANCE OF γ M-GLOBULIN IN DIFFERENT SPECIES

Species	Earliest γ M-globulin (days post coitus)	Age equivalent $k(t-t')$	References
Man	74	0.29	39
Pig	74	1.05	40
Guinea-pig	58	2.55	41
Rabbit	30	0.34	42
Rat	30	0.60	42
Mouse	24	0.35	42
Chicken	18	0.20	43

More information is available on the onset of antibody production (Table 3). Generally, the results in terms of equivalent ages agree very well. Antibody has been detected earliest in rabbits, mice and chicken, with the rat and opossum not far behind; all these animals produce antibody following antigenic stimulation at a similar physiological age: $k(t-t') = 0.36-0.58$. Active antiphage production in foetal lambs has been detected²⁸ 6 days after injection of phage after 35 days of gestation—one day before the first appearance of lymphocytes³. It is possible to predict from Table 3 that there may be a slightly earlier onset of the ability to produce antibody in cattle, pigs and hamsters as well as man (in view of his capacity to synthesize γ M-globulin at a much earlier age). Foetal dogs and guinea-pigs would clearly merit study. From Fig. 1 it can be predicted that cats become immunocompetent after about 38 days of gestation.

The application of age equivalence to the ontogeny of the immune response is clearly of great potential use. Not only does it make possible the unification of the onset of immunological function in a range of species, but it

can also be used to predict when animals will become immunocompetent in foetal or neonatal life. It is important, however, that such mathematical calculations are closely related to morphological events in development such as the first appearance of small lymphocytes and the time of peak growth velocity.

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Table 3. EARLIEST DETECTABLE ANTIBODY PRODUCTION

Species	Antibody observed (days post coitus)	Age equivalent $k(t-t')$	References
Man	112	0.78	44
Monkey	70	0.55	45
Cattle	118	1.00	*
Sheep	66	0.74	46
Pig	74	1.05	40
Dog	56	2.45	47
Opossum	28	0.58	48
Guinea-pig	67	3.20	49
Rabbit	31	0.36	50
Rat	28	0.50	51
Mouse			
NZB	27	0.44	52
Balb/c	29	0.52	52
Hamster	26	1.10	53
Chicken	24	0.40	54

* Personal communication from K. L. Fennestad and C. Borg-Petersen.

Prolongation of Skin Allograft Survival with Spleen Extracts and Antilymphocytic Serum

by

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A single dose of splenic extract is sufficient to induce a high degree of specific unresponsiveness to skin allografts in mice if used in conjunction with antilymphocytic serum. The interval between giving the extract and the antiserum is of great importance, and the unresponsiveness appears to be due to tolerance rather than enhancement.

ONLY the induction of specific unresponsiveness is likely to solve the immunological problems of organ transplantation. Because it is well known that, experimentally, tolerance is established much more readily if the antigenic disparity between donor and recipient is relatively small it seems reasonable to suppose that the progress now being made in tissue typing¹ will facilitate the induction of tolerance in organ recipients, provided that suitable methods become available.

It has been shown that unresponsiveness occurs if thymectomized mice treated with antilymphocytic serum (ALS) are injected with very large doses of lymphoid cells², and in a careful series of experiments Lance and Medawar³ have demonstrated that mice with intact thymuses become tolerant after administration of ALS in the first week after grafting and subsequent inoculation of as few as 25×10^6 F₁ hybrid spleen cells. Although these experiments clearly establish the principle that ALS can be used to potentiate tolerance induction, the use of living cells is clinically undesirable: cells most readily available are lymphoid and the recipient is liable to become the victim of a graft versus host reaction^{4,5}. The experiments described here were carried out to determine in mice the conditions in which tolerance induction with cell free extracts can be potentiated by ALS.

Spleen extracts consisting largely of sedimentable membrane fragments are immunogenic when injected intraperitoneally into mice differing from the tissue donor at the "strong" H-2 histocompatibility locus, that is they bring about the accelerated rejection of skin allografts⁶. Given intravenously these extracts are far less immunogenic and may even prolong the life of skin grafts by a few days, but in an H-2 compatible ("weak") donor-recipient combination their administration, by any route, can bring about substantial prolongation⁷. Martinez *et al.*⁸, using somewhat similar extracts, demonstrated that prolonged treatment of mice could extend graft survival: good results were obtained when the antigenic differences between donor and recipient were confined to the male-specific antigen, while in strong combinations only a fairly small effect was observed (three out of seventeen mice having exhibited "acceptance"). We chose to use a relatively crude and simply prepared kind of extract because, for tolerance induction, it is important that there should be no serious loss of antigenic specificities during extraction.

The extraction procedure was based on that described by Medawar^{7,9}, but differed in detail. About 5 g of splenic tissue was pressed through a stainless steel sieve and taken up in 25 ml. of 0.15 M phosphate-buffered saline (PBS), pH 7.4. The suspension was centrifuged at 500g for 10 min and the sediment resuspended in 5 ml. of PBS, to which 2 volumes of distilled water were added. The material was carefully mixed and sedimented at 3,000g for 10 min, and

it was resuspended in 15 ml. of distilled water. Resuspension was achieved by cutting up the gelatinous sediment with scissors, followed by pipetting. After a further spin at 3,000g for 10 min the supernatant was discarded and the sediment resuspended in 7 ml. of distilled water. This material was blended for 6 min in an Ultra-Turrax blender (TP 18/2, Scientific Instruments Ltd), the speed (approximately 10,000 r.p.m.) being determined by position 80 of a variable voltage transformer ('Variac', Gallenkamp). The concentration of salt was now restored to normality by adding 2 M saline and the material was blended for a further 2 min. After centrifugation at 3,000g for 15 min the supernatant was removed and PBS was added to it to give a concentration of 390 mg wet weight of spleen/ml. The pH was increased to 7.4 by the addition, drop by drop, of 0.1 N NaOH (the extract was magnetically stirred) and, finally, heparin (Heparin BP, Boots) was added to give a concentration of 14 U/ml. As the standard dose of this "semi-soluble" extract (SSE) was the equivalent of 250 mg wet weight spleen, mice were injected intravenously with 0.71 ml. containing 10 U of heparin. Extraction was carried out entirely in the cold, and special care was taken to prevent heating when blending. When required, extracts were stored in sealed ampoules at -80°C .

Two pools of ALS were prepared in New Zealand white rabbits using essentially the method of Levey and Medawar¹⁰. Lymph node cells from CBA mice were generally used and care was taken to minimize the degree of contamination with red blood cells. The sera, which were heated to 56°C for 30 min, Seitz-filtered and stored at -20°C in small aliquots, were virtually non-toxic even

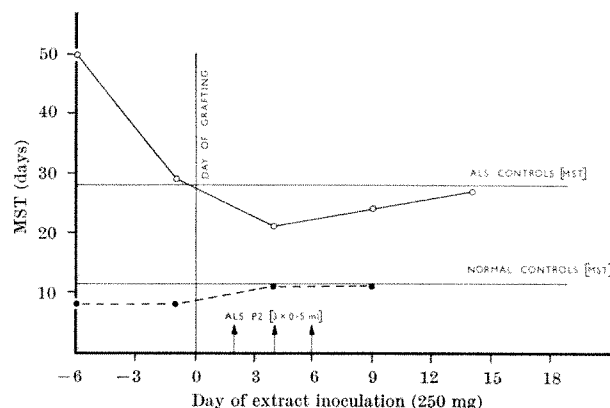


Fig. 1. Effect of a single dose of strain A spleen extract (250 mg wet weight spleen) on the median survival time (MST) of strain A skin grafts. The extract was inoculated intravenously into groups of ten CBA males at various times before and after transplantation, with and without ALS (P2). ○—○, Extract+ALS; ●---●, extract only.

when 0.5 ml. was injected intravenously into mice, and absorption with mouse red cells was therefore obviated. Both serum pools had cytotoxic antibody titres exceeding $1:10^4$, but there was some difference in their *in vivo* action in prolonging skin graft survival. ALS (0.5 ml.) was given intraperitoneally on days 2, 4 and 6 after transplantation of a skin allograft.

Induction of Specific Unresponsiveness

Two combinations of inbred mouse strains were used: A→CBA (strong) and C3H→CBA (weak). The median survival times (MST) of grafts transplanted to untreated recipients were 11 and 21 days, respectively; all recipients were 10–16 week old males. The technique of skin grafting was that of Billingham and Medawar¹¹.

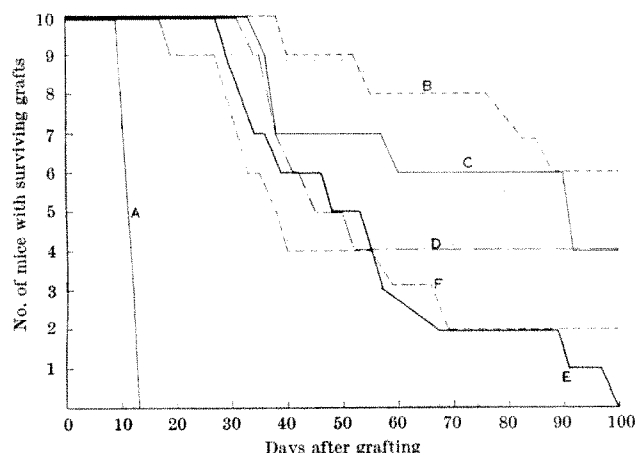


Fig. 2. Effect of a single dose of strain A extract inoculated intravenously into CBA males at various times before transplantation of strain A skin and ALS (P2) treatment. ALS (0.5 ml.) was given intraperitoneally on days 2, 4 and 6 after grafting. No treatment (A). Extract inoculated before grafting: 16 days (B); 11 days (C); 6 days (D) and 1 day (E). ALS only (F).

Figs. 1 and 2 give the results of two series of experiments in which SSE prepared from strain A mice was injected into CBA males at various times in relation to skin transplantation. It is apparent (Fig. 1) that SSE inoculation immediately before and after grafting was inefficacious but that a substantial shift in the MST occurred when SSE preceded grafting by 6 days. In another experiment (Fig. 2) pretreatment on day 6 failed to alter the MST although there were more long term survivors, but when the interval was lengthened to 11 days the MST went up to 90 days. By far the best results were obtained with an interval of 16 days, with an MST exceeding 120 days. The six mice in the 16 day group bearing healthy grafts on day 100 received a second strain A graft, and all these were accepted for a minimum of 24 days. Most went on for much longer, and two animals carried their grafts for a further 100 days, when the experiment was terminated. It is worth noting that, when rejection did occur in the other mice, both old and new grafts were affected at roughly the same time.

The longest interval between SSE inoculation and skin grafting investigated so far is 26 days, and the results were comparable with those obtained at 16 days. When experiments with 16 and 26 day intervals were performed in the weak combination, more than 50 per cent of the recipients carried their grafts for more than 150 days, and there is every indication that most of these grafts will be tolerated indefinitely.

In further experiments SSE was routinely injected 16 days before test grafting. The effect was found to be dose-dependent in the strong combination: the extract

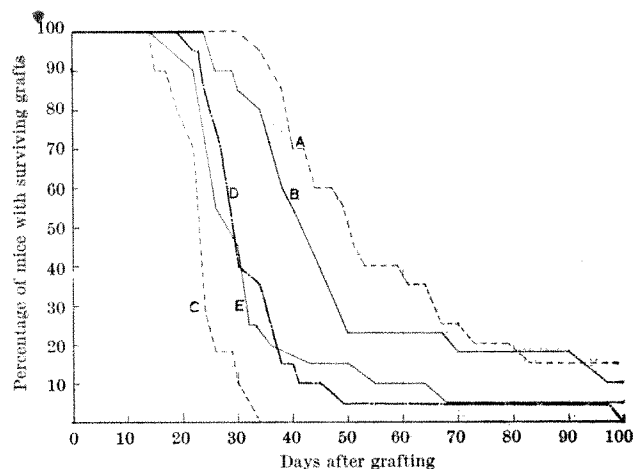


Fig. 3. Dose effect of strain A spleen extract inoculated intravenously into CBA males (18–20 mice/group) 16 days before transplantation of strain A skin and ALS (P2) treatment. The extract dose is expressed as mg wet weight spleen: A, 250 mg; B, 25 mg; C, 2.5 mg; D, 0.25 mg; E, ALS only.

from 25 mg still produced a significant effect, whereas 0.25 mg did not (Fig. 3). This experiment also seems to suggest that a dose of 2.5 mg actually undermined, if to a slight extent, the activity of the ALS. In the weak combination the dosage effect was rather less clear cut and good results were obtained with a dose as low as 2.5 mg.

SSE induced unresponsiveness is strain specific. This was demonstrated by two kinds of experiments: (a) syngeneic (CBA) SSE did not prolong the life of strain A grafts (Fig. 4), and (b) the unresponsiveness evoked by strain A SSE did not extend to Balb/c skin grafts (Table 1) although Balb/c mice are thought to carry only a single H-2 antigen absent in strain A.

Possible Mechanisms

The relationship between the time of antigen administration and skin grafting raises the possibility that the unresponsiveness is akin to immunological enhancement,

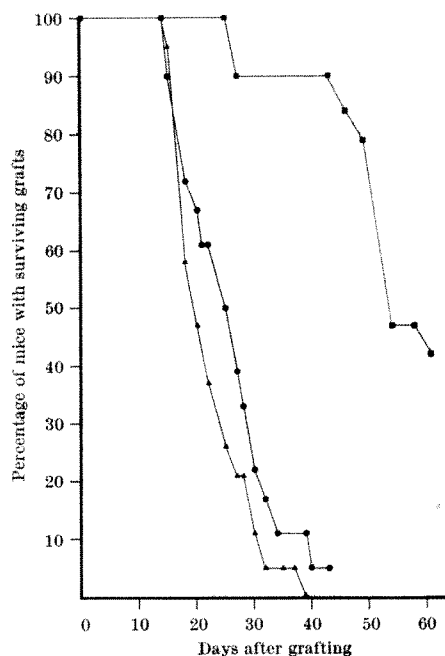


Fig. 4. Specificity experiment I: comparing intravenous inoculation of strain A and CBA extracts and heparinized saline into groups of nineteen CBA males 16 days before transplantation of strain A skin and ALS (P3) treatment (0.5 ml. on days 2, 4 and 6). ■, A extract; ▲, CBA extract; ●, heparinized saline.

Table 1. SPECIFICITY EXPERIMENT II: INTRAVENOUS INOCULATION OF CBA MALES WITH STRAIN A SPLEEN EXTRACT 16 DAYS BEFORE TRANSPLANTATION OF STRAIN A OR Balb/c SKIN, FOLLOWED BY 0.5 ml. OF ALS (P3) ON DAYS 2, 4 AND 6

Extract	No. of mice	Skin graft	Median survival time (days)
Strain A	5	Strain A	> 50
Nil	7	Strain A	24
Strain A	6	Balb/c	21
Nil	6	Balb/c	22

especially in view of the report¹² that rat kidney allografts can be protected by passive transfer of hyper-immune serum. To sustain such a hypothesis it would be necessary to demonstrate that SSE-injected mice form humoral antibodies against donor strain antigens in adequate amounts. We therefore examined the sera of ten mice at various times before and after skin transplantation for the presence of haemagglutinating antibodies, using the polyvinyl-pyrrolidone technique. The results were uniformly negative, even when some of the grafts were undergoing protracted rejection between 40 and 60 days. (The one possible exception was an animal which gave a dubious titre of 1:8 on day 50, when its skin graft was being rejected.) These negative findings are supported by tests for haemagglutinating¹³, cytotoxic¹⁴ and antigen-binding¹⁵ antibodies on the pooled serum prepared from SSE-injected mice 21 days after skin grafting, at a time when they all bore perfect skin grafts and the grafts on ALS control animals were close to their MST. These tests were again totally negative. Our results are therefore not compatible with an enhancement hypothesis.

Sensitization *per se* is not sufficient to explain the effect. When SSE was substituted by a skin graft, or by lymph node cell suspensions, no comparable effect was obtained (Table 2). Indeed, skin grafts and the higher cell doses completely subverted the action of the ALS, and while this was not true for doses of 10^5 and 10^4 cells none of them raised the MST above that of the ALS controls. Because even 10^4 cells constituted a sensitizing dose in normal recipients experiments with even lower doses are in progress; but at present we must conclude that the physical form of the antigen is vitally important and that only subcellular extracts provide the correct stimulus for specific unresponsiveness to be induced in experiments of this design. It is of considerable interest that liver extracts have been found to have very similar properties, despite the fact that when prepared from perfused livers they are totally non-immunogenic (unpublished work of L. B. and J. A. Hansen).

Several recent publications have been concerned with the possible synergistic effect of spleen extracts and ALS; that only relatively small effects have been uncovered is likely to be due to the fact that the extracts were administered too near the time of skin transplantation. Thus Abbott, Monaco and Russell¹⁶ commenced a prolonged treatment with extracts 1 day before grafting and encountered substantial prolongation of graft survival only in thymectomized mice. Lance and Medawar⁹ found that a single pulse of SSE had no discernible effect when given

in conjunction with ALS, and even when SSE was administered repeatedly every two weeks the MST was not significantly increased (though a small proportion of these mice tolerated their grafts for periods well in excess of the controls).

Assuming that ALS acts on the pool of recirculating lymphocytes (for recent review see ref. 17) and that SSE does not cause cellular proliferation in the central lymphoid organs (an assumption that is being investigated), two possible explanations are suggested to account for our observation that SSE acts more effectively when given well before skin grafting and ALS treatment. First, SSE may mobilize antigen-specific cells from the central lymphoid organs, so that a greater proportion of these cells is eliminated by the ALS; and second, SSE may act on antigen-specific cells by making them tolerant and/or short lived. Both hypotheses, which are by no means mutually exclusive, would explain the time-dependence of the phenomenon.

Clinical Applications

It would be premature to apply this method of inducing specific unresponsiveness to human organ recipients until further experiments have been carried out in species other than rodents and with organ grafts, and before the efficacy of more highly purified extracts has been ascertained. It is, however, encouraging that a synergistic action of tissue extracts and conventional immunosuppressive drugs prolonging the life of kidney allografts in dogs has already been demonstrated¹⁸. The method described here is an advance in that it requires only a limited course of ALS treatment (in clinical terms the total dose of ALS used so far is nevertheless very high) and a single injection with extract (though we have shown with liver extracts that in an H-2 incompatible combination the results can be substantially improved if in addition smaller doses are injected for some time after transplantation (unpublished work of L. B. and J. A. Hansen)). Clinically, the use of liver as opposed to spleen extracts could be advantageous not only because much larger amounts of antigen would be obtained, but also because of the non-immunogenicity of liver extracts (unpublished work of L. B. and J. A. Hansen). The problem of pre-treating patients can probably be overcome by storage of the organ (this is as yet impracticable in most cases) by selection of separate extract and organ donors closely matched for their HL-A antigens, or by using stored extracts covering all major antigens.

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Table 2. EFFECT OF EXPOSING CBA MALE MICE TO STRAIN A SKIN GRAFTS OR LYMPH NODE CELLS 16 DAYS BEFORE SKIN GRAFTING, WITH 0.5 ml. OF ALS GIVEN ON DAYS 2, 4 AND 6 AFTER GRAFTING

Antigenic stimulus	ALS	No. of mice	Median survival time (days)
Skin graft	P2	20	< 10
5×10^4 cells*	P2	19	< 10
None	P2	16	30
10^4 cells	P3	9	10
10^5 cells	None	10	< 8
10^6 cells	P3	9	14
10^7 cells	None	10	< 8
10^4 cells	P3	10	10
10^5 cells	None	10	< 8
None	P3	10	18

* Injected intraperitoneally; all other cell suspensions were given intravenously.

Rejection of Skin Allografts by Radiation Chimaeras: Selective Gene Action in the Specification of Cell Surface Structure

by

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Use of the technique of acquired tolerance suggests that epidermal cells contain distinctive transplantation allo-antigens—antigens not also possessed by marrow and lymphoid cells.

SEVERAL cell surface alloantigens of the mouse, coded by several independent genetic loci, are expressed exclusively or predominantly on thymocytes and lymphocytes; they have been referred to as differentiation antigens¹. A similar alloantigenic system characteristic of immunoglobulin-forming cells has also been described². One important implication of these newly recognized antigenic systems is that they may aid in the understanding of the way in which cells identify one another and so assemble themselves systematically into biological structures ("cell recognition"). This can be readily envisaged in genetic terms. Selective gene action determines the different patterns of protein synthesis in differentiated cells, and so it should also account for the specialization of cell surface structure, and that for the characteristic properties of surface recognition which cells acquire as a result of differentiation, and which must underlie all forms of multicellular organization.

To strengthen the hypothesis that the programme of differentiation for each cell type includes the genetic specification of a characteristic surface conferring specific mutual cell recognition, we have looked for evidence of differentiation antigens in cells other than those of the lymphoid system. The serological methods which revealed these antigens on lymphoid cells¹ require dispersed viable cell populations for testing with cytotoxic antisera, a technique that is not readily applicable to the cells of tissues such as skin. Although appropriate serological procedures can no doubt be devised, we decided to look first for skin differentiation alloantigens *in vivo*. Our experiments were suggested by reports that chimaerism in man, cattle and mice (and, according to a recent report, in rats also³) does not always guarantee the survival of skin allografts from the donor whose haemopoietic cells continue to colonize the recipient⁴. In other words, immunological tolerance⁵ of haemopoietic cells does not invariably entail permanent tolerance of skin grafts from the same donor, as it might be expected to do if there were no organ-specific alloantigens capable of eliciting a homograft reaction⁶. Analogy with the differentiation antigens of lymphoid cells⁷ prompted the explanation that these rejections were caused by an immune response to alloantigens expressed on the skin of the donor but not on the donor's haemopoietic cells^{7,8}.

We studied chimaeras formed by protecting lethally irradiated mice with bone marrow or spleen cells because

in chimaeras of this type the recipient's haemopoietic system is totally replaced by the donor's (as far as this can be determined), making it easy to prove chimaerism by typing red cells⁹ or lymphocytes¹⁰ for donor H-2 antigen. Our choice of the combination (C57Bl/6 × A)F₁ donor and C57Bl/6 recipient (*abbr* C57) was based on reported observations that A strain recipients that had been inoculated at birth with (CBA × C57)F₁ cells rejected C57 skin but not CBA skin ("split tolerance")¹¹. Accordingly, strains C57 and A (but not CBA and A) can be considered to carry different alleles at a locus specifying a differentiation alloantigen of skin. The purpose of using F₁ rather than A strains donors of spleen and bone marrow cells was to avoid graft versus host reactions. Fig. 1 summarizes the results of our several experiments with this donor-recipient combination.

Course of Rejection

The twenty-three mice in group 1 received A strain skin grafts 11 weeks after irradiation. They rejected them about as rapidly as if there had been H-2 incompatibility. The rejection of second-set A strain and (C57 × A)F₁ skin grafts in the same animals was accelerated. Later, all these mice were shown to be chimaeric by H-2 haemagglutination and by cytotoxic tests on lymphocytes obtained by excising an inguinal lymph node under sodium pentobarbital anaesthesia (Table 1). In these tests, and all other such tests we have carried out on radiation-chimaeras of this type, both in these and other experiments, it has been impossible to detect any erythrocytes or lymphocytes of recipient type. Thus, although it cannot absolutely be excluded that rejection was brought about by a small undetectable fraction of immune cells of recipient origin, it seems more likely that the cells responsible were donor cells that had lost their immunological tolerance of A strain skin alloantigen after being transferred to C57 recipients which had a different skin alloantigen allele. We infer that maintenance of self-tolerance to skin alloantigen was lost when the immunocompetent cell population was removed from the environment where this antigen is normally present (compare ref. 12).

Each of the ten mice in group 2 was restored with spleen cells from a single (C57 × A)F₁ splenectomized donor which then provided a skin graft for the same recipient 5 weeks later. The rejection of these grafts shows that

Table 1. H-2 TYPING OF ERYTHROCYTES AND LYMPHOCYTES OF CHIMAERAS

	Haemagglutination test ^a						Cytotoxic test ¹⁰			
	H-2 ^b anti H-2 ^a 1:16	H-2 ^b anti H-2 ^a 1:64	H-2 ^b anti H-2 ^a 1:256	H-2 ^b anti H-2 ^a 1:20	H-2 ^a anti H-2 ^b 1:80	H-2 ^a anti H-2 ^b 1:320	H-2 ^b anti H-2 ^a 1:40	H-2 ^b anti H-2 ^a 1:80	H-2 ^a anti H-2 ^b 1:40	H-2 ^a anti H-2 ^b 1:80
(C57 × A)F ₁ → C57 chimaeras*	+++	+++	+++	+++	+++	+++	> 95	> 95	> 95	> 95
Normal control mice	+++	+++	+++	+++	+++	+++	> 95	> 95	> 95	> 95
(C57Bl/6 × A)F ₁	++	++	++	++	++	++	> 95	> 95	> 95	> 95
C57	++	++	++	++	++	++	< 10	< 10	< 10	< 10
A	+++	+++	+++	++	++	++	> 95	> 95	< 10	< 10

* Every irradiated C57 mouse restored with (C57 × A)F₁ cells and tested with H-2 antisera by haemagglutination or cytotoxicity for the presence of donor cells gave the results shown here, which are indistinguishable from the normal (C57 × A)F₁ control. Thus erythrocytes and lymph node lymphocytes of recipient origin were either absent or were present in numbers too few to be detected.

The usual serological controls, omitting antibody or complement, were invariably negative. As a further test of serological specificity, cytotoxic tests were performed with the serum (C57 × A)F₁ anti C3H (anti H-2.32); reaction with lymph node lymphocytes of normal H-2^k mice was strongly positive, but there was no reaction with lymphocytes of the chimaeras, indicating that the latter are not abnormally sensitive to non-specific lysis by mouse serum and guinea-pig serum (source of complement).

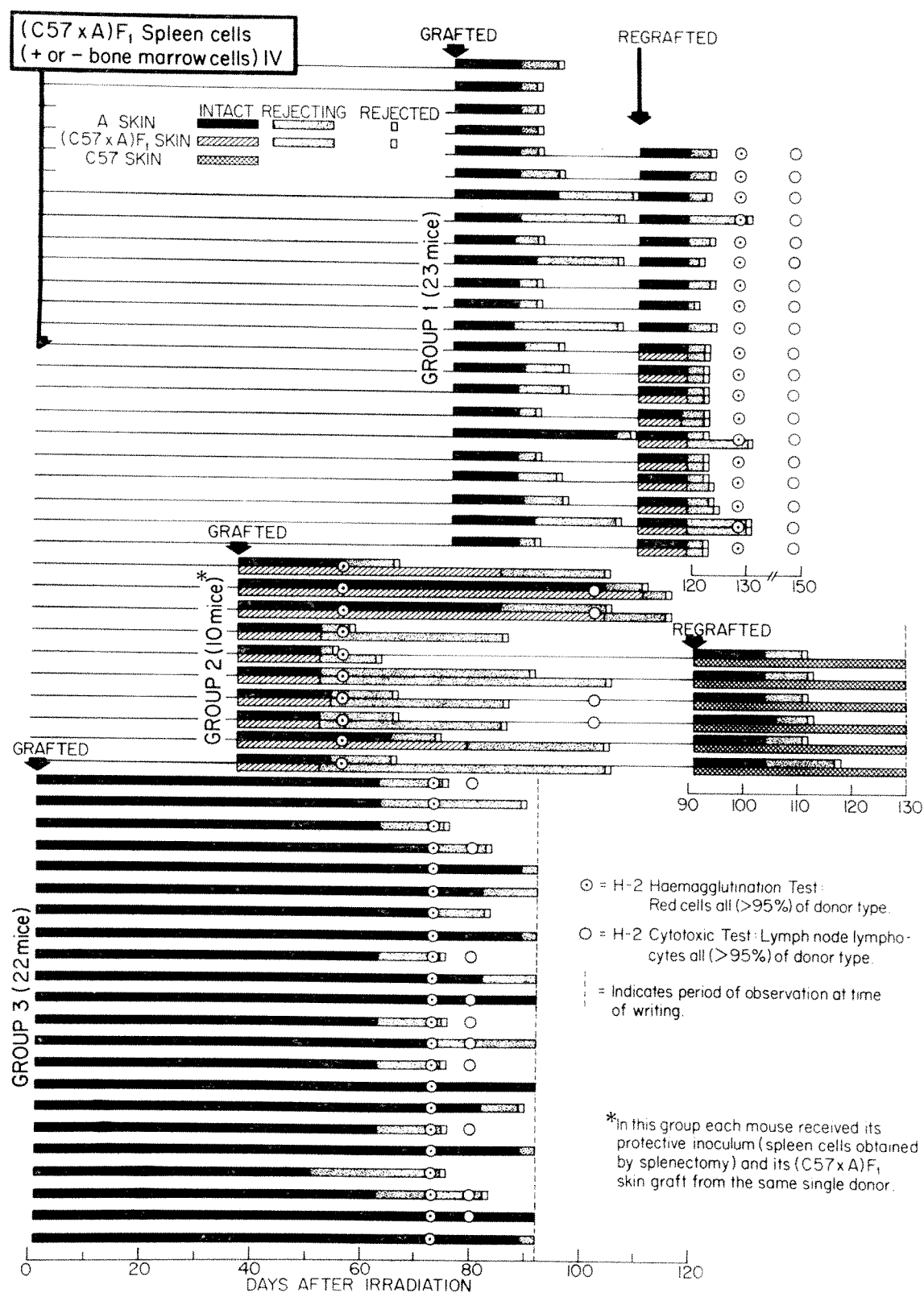


Fig. 1. Summary of experiments in which lethally irradiated C57 mice, restored with (C57 x A)_F₁ haemopoietic cells, were grafted with A strain and (C57 x A)_F₁ skin. Protective inocula (given intravenously within 24 h of irradiation) were: for group 1, pooled bone marrow and spleen cells (5×10^7 /mouse); for group 2, each mouse received (2 to 6×10^6) spleen cells (obtained by splenectomy) from a single (C57 x A)_F₁ donor, which also provided the (C57 x A)_F₁ skin graft for the same individual recipient 5 weeks later; for group 3, pooled spleen cells (5×10^7 /mouse). In group 1 the recipients, and the donors both of haemopoietic cells and of skin, were females. In groups 2 and 3, all were males. Total body irradiation (750 r) was delivered from a 'Maxitron 300' (General Electric) X-ray machine (HVL 2 mm Cu); control irradiated mice receiving no protective inoculum invariably died within 30 days. Terramycin (Pfizer) was included in the drinking water, and Kanamycin (Bristol Laboratories) was administered intramuscularly, for 3 weeks after irradiation. Skin from the tail was grafted by the method of Billingham and Medawar¹⁴; double grafts, A and (C57 x A)_F₁ were placed side by side in the same bed. All mice were obtained from our own breeding colonies (origin: C57Bl/6J and A/J); recipients were 10-13 weeks old at the time of irradiation.

rejection cannot be explained by genotypic differences between the donors of the skin grafts and the donors of the cells producing chimaerism. We infer that the antigenic difference between haemopoietic cells and skin cells is phenotypic and is a consequence of selective gene action¹.

The twenty-two mice in group 3 were grafted with A strain skin within 24 h of irradiation, shortly after receiving their protective inoculum of spleen cells. The survival of these grafts was considerably longer than that of grafts in groups 1 and 2 where grafting was delayed for 11 and 5 weeks respectively. On present evidence it is not possible to say whether this is due to immunosuppression following irradiation, or whether several weeks is required for the expanding population of donor cells to lose its tolerance of the A strain skin alloantigen from which it is separated. In any case, there was rejection, showing that a skin graft was not sufficient to maintain the presumed initial tolerance of the donor population. Possibly immediate grafting postpones loss of tolerance, for the prolongation of graft survival in group 3, in comparison with group 2, was more than the 5 weeks which elapsed between irradiation and skin grafting in group 2. But groups 2 and 3 are not strictly comparable because they were irradiated on different occasions, the former received two grafts and the latter one, and they received different numbers of spleens. The idea that early grafting partially sustains tolerance is therefore no more than a suggestion to be pursued in further experiments.

The serum of chimaeras that rejected A or (C57 × A)F₁ skin grafts has been tested stringently for cytotoxic antibody to normal A strain thymocytes or lymphocytes with negative results.

In other experiments lethally irradiated A strain female mice were restored with (C57 × A)F₁ female bone marrow and spleen cells, and were grafted 11 weeks later with C57 female skin. Survival of the grafts was much longer, and rejection more protracted, than in the foregoing experiments; ten out of twenty-one of the recipients (proven chimaeras) rejected C57 skin within 170 days of grafting. In another donor-recipient combination, (BALB/c × DBA/2)F₁-to-DBA/2, five out of twenty-one recipients (similarly irradiated and restored, but not tested for chimaerism) rejected BALB skin grafts within 250 days.

The explanation proposed for all these rejections may apply also to cases of late rejection of skin allografts

which have been attributed previously to loss of chimaerism with increasing age¹³. Similarly, in the case of donor-host combinations where inoculation of haemopoietic cells into neonatal recipients characteristically fails to induce tolerance of donor skin grafts, it may be that the explanation lies in a strong skin alloantigen, rather than in failure to establish chimaerism.

The special value of the (C57 × A)F₁-to-C57 combination is the feasibility of securing skin graft rejection in 100 per cent of chimaeric recipients. Thus the genetic experiments required to define the locus or loci responsible for specifying skin alloantigen will be greatly facilitated.

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Immunosuppressive Effects of Soluble Cell Membrane Fractions, Donor Blood and Serum on Renal Allograft Survival

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Soluble cell membrane extracts of liver and spleen have been injected intravenously at the time of renal allografting. In dogs and rhesus monkeys there was slight prolongation of transplant survival in some experiments. Survival was markedly prolonged in 30 per cent of pigs, however. Donor blood and serum had a similar effect in protecting porcine liver allografts from rejection.

LIVER allograft rejection has been reported in dogs^{1,2}, rats³, pigs⁴⁻⁷, rhesus monkeys⁸ and man^{7,9}, but in each of these species livers are rejected less vigorously, or can be retained more easily with immunosuppressive agents, than are allografts of other organs and skin. In our

experience orthotopic liver allograft rejection in the pig has been mild or minimal in animals given no immunosuppressive treatment observed up to 2.5 years¹⁰⁻¹². We have transplanted livers orthotopically between litter mates, within strains of pigs which have been partially

line bred, and across three distinct breeds—large white landrace and wessex saddleback. In each case liver allograft rejection consisted of no more than slight infiltration of the portal tracts with mononuclear cells and a variable increase in fibrous tissue. There have been no severe destructive lesions of the hepatic parenchyma. We have been unable to detect recipient lymphocyte entrapment in liver allografts, nor release of large amounts of enzymes from the liver. Postoperative concentrations of cortisol and corticosterone in the serum have been normal. It is our impression that a short operating time and minimal hepatic ischaemia correlate directly with less histological evidence of rejection. In marked contrast similar categories of pigs usually react vigorously against skin, heart and kidney allografts producing destructive rejection between 5 and 10 days. Mixed lymphocyte culture reactions between such pigs are generally positive¹³, with the exception of tests between 25 per cent of litter mates¹⁴. In some experiments, after liver allografting, specific long lasting depression of the mixed lymphocyte reaction of recipient cells against the donor was observed, a pattern that would be expected in a tolerant animal^{15,16}. In other experiments stimulation persisted at the same or an increased level without the organ being rejected¹³. If the *in vitro* proliferative response parallels *in vivo* immune mechanisms, then stimulation failing to lead to organ rejection could be a consequence of an ineffective cellular response or protection of the graft by enhancing antibody. Partial tolerance and enhancement are not necessarily mutually exclusive¹⁷⁻¹⁹.

Both orthotopic and auxiliary liver allografts confer marked protection from rejection on simultaneously transplanted kidneys from the same donor. This renal allograft protection has persisted after removal of the auxiliary liver 24 h after transplantation¹². Because liver allograft protection from rejection has always been greater for tissues from the same donor than from third party animals, transplantation antigens must be involved. Usually third party grafts are rejected at the expected time, although occasionally survival has been prolonged. Chance sharing of transplantation antigens between the third party animal and the liver donor and/or the recipient is a possible explanation. In view of the variability of third party graft survival, antigen sharing would seem to be more likely than an additional non-specific effect of the grafted liver, although there is evidence of non-specific protection in classical tolerance²⁰.

We have suggested that the allografted porcine liver can induce partial immunological tolerance in animals with competent immune systems¹². The initial effect is too rapid for antibody production, but a later contribution of specific enhancement has not been excluded. It is possible that the porcine liver produces transplantation antigens in a tolerogenic form¹¹. In sympathy with this hypothesis was the demonstration of HLA antigens in the albumen fraction of human serum by Van Rood²¹. We have repeated and confirm these findings and have made similar observations in the pig (unpublished observations of D. W. and V. C. J.). Preliminary investigations suggested that soluble liver cell membrane preparations in the pig had immunosuppressive activity¹². The experiments reported here were designed to investigate this hypothesis further. The effects of soluble cell membrane extracts from liver and spleen on renal allograft survival have been studied in the pig, dog and rhesus monkey. In pigs further observations have been made on animals with renal allografts treated with donor blood or serum.

Preparation of Antigen

Fresh spleens and livers were separately disaggregated by passing through a mechanical tissue grinder and then gently homogenized to give cell suspensions. These were extracted by hypotonic lysis²², and the cell residue was separated from the extracted material by centrifuging at

500g. This supernatant was centrifuged at 85,000g for 90 min and the deposit resuspended at 2 per cent in water saturated with thymol and stored at 4° C. This was a crude membrane fragment preparation.

Soluble antigen was obtained by incubation of this material at 37° C in 0.05 M Tris buffer, pH 7.5, for 2.5 h and centrifuging at 150,000g for 2 h. The supernatant was concentrated to one-tenth of its volume and put aside. The sediment was resuspended at 2 per cent w/v and incubated with papain (activated with cysteine) at 37° C for 60 min; this was cooled, sodium iodoacetate added as inhibitor, and centrifuged at 150,000g for 2 h. The supernatant was concentrated and passed through a 'Sephadex G-75' column to remove papain. The excluded fraction was added to the supernatant previously obtained following incubation with Tris buffer alone. The pooled material was adjusted to 10 mg/ml. of protein, passed through a 'Millipore' filter (3 µm) and stored at -50° C until used.

This kind of material has been referred to as "crude soluble antigen" and, as such, was used in most of the experiments described. For a few experiments crude soluble antigen was purified by passing through 'Sephadex G-200' columns, where the intermediate (retarded) fraction was used. This fraction contains H-2 or HLA active material when this procedure is applied to mouse or human material respectively²³⁻²⁵.

Surgical Technique

Pigs weighing 30-40 kg, of either sex, were used as recipients; donor animals were larger. Under halothane anaesthesia with spontaneous respiration the abdominal cavity was opened and the animals were heparinized. The left kidney of the donor was transplanted orthotopically to the bed of the recipient's left kidney after nephrectomy. The renal artery with a Carrel patch of aorta was anastomosed to the side of the recipient's aorta. The renal vein and ureter of donor and recipient were joined end to end²⁶. Penicillin (300 mg) and streptomycin (0.5 g) were given by intramuscular injection and 500 ml. of normal saline was infused into a neck vein. No drugs were given postoperatively.

Experimental Groups

(1) Control renal allografts were given to thirty-one animals. Seventeen had both their own kidneys removed at the time of grafting and died after between 5 and 33 days (Fig. 1). Ten of the thirty-one grafts were between litter mates, and nine of these were moderately to severely rejected. One allograft was normal at autopsy after 12 days. The other twenty-one recipients died or had their allografts removed after between 6 and 25 days. All of these grafts had substantial to severe rejection. The relationships of donor and recipient were: three half-sibs,

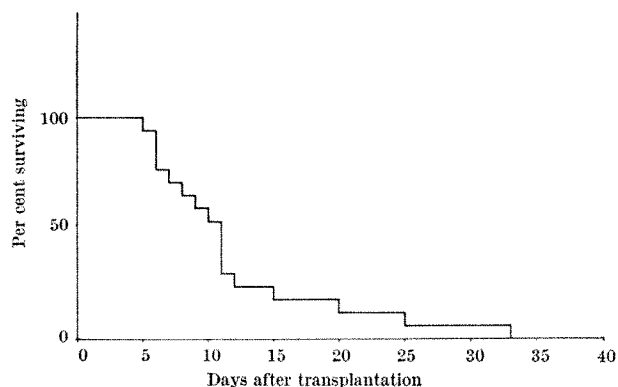


Fig. 1. Survival of seventeen bilaterally nephrectomized pigs with renal allografts. Nine transplants were between litter mates. These recipients survived 5, 7, 9, 10, 11, 12, 15, 20 and 33 days.

one distant relative, eight unrelated pigs and nine of a different breed.

(2) Seventeen of the bilaterally nephrectomized pigs with renal allografts treated with soluble cell membrane extracts at the time of operation (Table 1) survived beyond 4 days (Fig. 2). None of these transplants were between litter mates. Eight pigs died after 8–29 days with rejected kidneys. The relationships of donor and recipient were five distant relatives, two unrelated and one of a different breed. Five animals with unrelated donors died after 36–156 days with rejected kidneys. Four were alive after 105–258 days. The relationships of donor and recipient were one half-sib, one distant relative and two unrelated.

Table 1. BILATERALLY NEPHRECTOMIZED PIGS WITH RENAL ALLOGRAFTS TREATED WITH SOLUBLE CELL MEMBRANE EXTRACTS

Expt.	Survival (days)	Donor relationship	Type	Antigen dose (mg)	Route
1	8	Different breed	Liver + spleen	5,300	Intraportal†
2	8	Unrelated	Liver + spleen*	319	Intravenous
3	9	Distant	Spleen	625	"
4	9	Distant	Liver	1,200	"
			Spleen†	291	"
5	10	Unrelated	Liver + spleen§	4,210	"
6	17	Distant	Liver	686	"
7	19	Distant	Liver	123	"
			Spleen†	23	"
8	29	Distant	Liver	5,000	"
9	36	Unrelated	Liver	5,000	"
10	48	Unrelated	Liver + spleen	2,700	Intraportal†
11	100	Unrelated	Liver	5,744	Intravenous
12	105A	Half-sib.	Liver + spleen	3,042	Intraportal†
13	112	Distant	Liver	1,404	Intravenous
			Spleen†	695	"
14	154A	Unrelated	Spleen	742	"
15	156	Unrelated	Spleen	523	"
16	161A	Distant	Liver	87	"
			Spleen†	83	"
17	258A	Unrelated	Liver¶	1,700	"

* Crude soluble antigen from the kidney donor was used unless specified to the contrary.

A. Alive.

† Purified soluble antigen.

‡ Additional spleen antigen from same donor given 7–8 days after transplantation.

§ Antigen infusion started during operation and continued for 24 h.

¶ Mixed pool of liver and spleen antigen from thirteen donors including kidney donor.

‡ Antigen donor different from kidney donor, both unrelated to recipient and to each other.

(3) Six bilaterally nephrectomized pigs with renal allografts treated with donor blood were given 750–1,000 ml. of donor blood intravenously during the operation (Fig. 3). Three died after between 7 and 10 days with rejected kidneys. The relationships of donor and recipient were two unrelated and one of a different breed. Three were alive after 78–85 days, and in these cases the relationships of donor to recipient were two unrelated and one of a different breed.

(4) Six bilaterally nephrectomized pigs with renal allografts treated with donor serum were given 300–500 ml. of donor serum intravenously during the operation (Fig. 3). Five died after between 8 and 29 days with rejected kidneys—the 29 day survivor was given a further

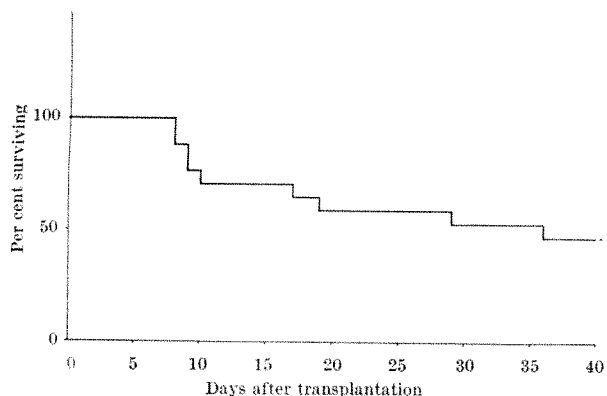


Fig. 2. Survival of seventeen bilaterally nephrectomized pigs with renal allografts treated with cell membrane extracts. None of the transplants were between litter mates.

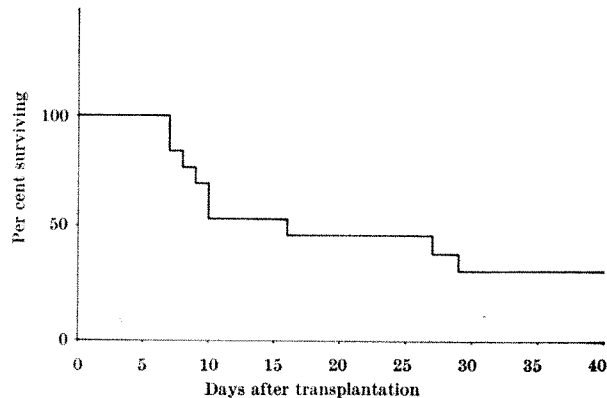


Fig. 3. Survival of twelve bilaterally nephrectomized pigs with renal allografts treated with donor blood or serum (six blood; six serum). None of the transplants were between litter mates.

500 ml. of donor serum at 7 days. The relationships of donor and recipient were two half-sibs and three distant relatives. One animal was alive after 91 days—its donor was unrelated.

(5) Fourteen bilaterally nephrectomized dogs, with renal allografts treated with soluble donor cell membrane extracts (Table 2), survived for 6–28 days. The allograft of the dog which died of pneumonia after 24 days was normal; all other kidneys were rejected.

(6) Seven bilaterally nephrectomized rhesus monkeys, with renal allografts treated with soluble donor cell membrane extracts (Table 3), died after 6–25 days. All kidneys were rejected.

Table 2. BILATERALLY NEPHRECTOMIZED DOGS WITH RENAL ALLOGRAFTS TREATED WITH SOLUBLE CELL MEMBRANE EXTRACTS

Expt.	Survival (days)	Type	Antigen dose (mg)	Route
1	6	Spleen	149	Intravenous
2	9	Liver	6,700	"
3	9	Liver	350	"
4	9	Liver	1,200	Intraportal
5	9	Liver	1,800	"
6	9	Spleen	630	"
7	11	Spleen	307	Intravenous
8	12	Spleen	342	"
9	14	Liver	625	"
10	15	Spleen	260	"
11	17	Liver + spleen	7,000	Intraportal*
12	21	Liver	1,600	Intravenous
13	24	Spleen	385	"
14	28	Liver	1,000	Intraportal

* Antigen infusion started during operation and continued for 24 h.

Table 3. BILATERALLY NEPHRECTOMIZED RHESUS MONKEYS WITH RENAL ALLOGRAFTS TREATED WITH SOLUBLE CELL MEMBRANE EXTRACTS

Expt.	Survival (days)	Type	Antigen dose (mg)	Route
1	6	Spleen	6	Intravenous
2	7	Spleen	10	"
3	9	Spleen	25	Intraportal
4	10	Liver	250	"
5	11	Spleen	25	"
6	11	Liver	250	"
7	25	Liver	61	Intravenous

Reasons for Little Rejection in Pig

Why we have found porcine orthotopic liver allograft rejection to be consistently so minimal is not clear. Minor technical points or the pig types we have used could be relevant, but in view of the conventional rapid rejection of other allografted tissues, the usually positive mixed lymphocyte culture reactions and the variety of pig breeds used, close histocompatibility between donor and recipient is an untenable explanation. An unlikely possibility is that certain strong transplantation antigens present in some strains of pigs are absent in the three breeds we have used. Even if this was the case, there is sufficient histoincompatibility between our pigs to produce aggressive rejection of all tissue so far allografted with the exception of the liver. Thus the behaviour of porcine hepatic allografts in contrast remains remarkable and must indicate a phenomenon of biological significance. More con-

sistent with the observations is the proposition that pigs vary in the amount and/or type of transplantation antigens produced by the liver. This might be influenced by environmental factors. It is not known if this is a unique property of the pig. Certainly the difference between liver and other organ allograft rejection in the dog, rat and several primate species is far less than in the pig^{1-3,7,9}. The 7 month survival, however, of one of our rhesus monkeys, which was given no immunosuppressive treatment, contrasts markedly with the behaviour of the other organ grafts, which in this species are usually rejected promptly⁸. Soluble transplantation antigens given intravenously possibly have more effective access to the lymphoid system in the pig than in other species because of peculiarities of porcine lymphocyte circulation and lymph node structure^{27,28}.

The immunosuppressive effect of soluble cell membrane extracts of donor liver and spleen has been unimpressive in the dog and rhesus monkey. In the pig, however, the prolongation of renal allograft survival has been comparable with that produced by a liver allograft. Specificity has not been investigated. In all but one experiment membrane extracts were prepared from the kidney donor. One pig survived nearly 9 months on a renal allograft from a donor unrelated to the animal from which the cell membrane extract was prepared. This does not exclude a specific mechanism, which could have resulted from sharing transplantation antigens between the membrane extract donor and kidney donor. Further studies with third party membrane extracts should help to clarify this point. There was no obvious difference in the effects of soluble cell membrane extracts prepared from liver or spleen, nor were the rate and route (intravenous or intraportal) of infusion apparently significant. A dose response relationship in terms of weight of protein and immunosuppressive effect was not established. The prolongation of renal allograft survival in animals treated with donor blood or serum is consistent with the proposition that soluble tolerogenic transplantation antigens can be present in normal pig serum.

These experiments on pigs are compatible with the hypothesis that soluble transplantation antigen can induce partial tolerance to renal allografts in immunologically competent pigs. It should, however, be emphasized that definitive proof is lacking. When well defined porcine tissue typing sera are available it should be possible to determine whether the cell membrane extracts do contain transplantation antigens and, if so, quantitative experiments could be performed. Then it would be

necessary to demonstrate that liver allografts release into the circulation similar soluble antigens capable of prolonging renal allograft survival.

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RNA Polymerase Mutants blocked in Sporulation

by

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A class of RNA polymerase mutants of *B. subtilis* fails to sporulate. The same mutational alteration prevents the change in template specificity of the enzyme characteristic of sporulating wild-type cells, suggesting that this change plays an essential part in the sporulation process.

It has recently been shown that after infection of *Escherichia coli* by phages T4 (refs. 1 and 2) specific changes occur in the protein subunits of RNA polymerase that determine template specificity of the enzyme. These changes are responsible for the expression of certain

phage genes and possibly for the turn-off of host genes. The discovery that the template specificity of RNA polymerase becomes altered during spore formation in *Bacillus subtilis*³ suggested a similar mechanism for the control of gene expression during the intracellular differ-

entiation that sporulation represents. We demonstrated that sporulation is accompanied by loss of ability of RNA polymerase to transcribe the DNA of phage ϕ *in vitro*, a finding consistent with the known inability of this phage to grow or to induce phage-specific enzymes in sporulating cells⁴. We proposed that this change in template specificity might control, at least in part, the switchover from expression of "vegetative genes" to expression of "sporulation genes"^{5,6}. It seemed possible that vegetative *B. subtilis* RNA polymerase would undergo structural modification, including alteration or replacement of a σ factor component; the resulting "sporulation" enzyme would be unable to transcribe either the phage ϕ DNA genome or bacterial genes coding for vegetative proteins, that is, proteins synthesized exclusively in growing cells. In confirmation of this hypothesis experiments described in the following article⁸ show that transcription of phage ϕ DNA *in vitro* is dependent on an RNA polymerase σ factor of molecular weight 57,000 and show that the change in template specificity during sporulation is accompanied by a modification in the structure of a β subunit of the enzyme. Kerjan and Szulmajster⁹ and Avila *et al.*¹⁰ also found that *B. subtilis* RNA polymerase depends on a σ factor for transcription of coliphage T4 DNA and *subtilis* phage ϕ 29 DNA, respectively.

The drug rifamycin and its analogue, rifampicin, are known to act directly on RNA polymerase from both *E. coli* and *B. subtilis*^{11,12}. Here we report that among rifampicin-resistant (*rfr*) mutants of *B. subtilis* there is a frequent class of mutants that, in a single mutational event, has gained drug-resistance *in vivo* and *in vitro* and lost the ability to sporulate. This mutation prevents the change in template specificity of RNA polymerase characteristic of sporulating wild-type cells, indicating that this change is a critical step in the sporulation process.

Isolation of *rfr* 10

We have shown earlier that the component of *B. subtilis* RNA polymerase determining sensitivity to rifampicin is conserved during sporulation³. This was concluded from a study of the properties of the mutant *rfr* 11, which is resistant to rifampicin *in vivo* and produces a mutant RNA polymerase that is drug resistant in extracts of both log-phase and sporulating cells. *Rfr* 11 is not impaired in spore production (phenotype *rfr*⁺ *sp*⁺; see Table 1).

A second RNA polymerase mutant, called *rfr* 10, was found to have gained resistance to rifampicin and simultaneously lost the ability to sporulate. *Rfr* 10, like *rfr* 11, was selected after ultraviolet mutagenesis of *B. subtilis* 3610 as a young colony able to grow on tryptose blood agar base (Difco) plates containing 5 μ g/ml. rifampicin (Ciba). When tested for sporulation by phase-contrast microscopy after growth for 24 h at 37° C in the standard conditions^{3,4}, only 5 per cent of the surviving cells contained spores as compared with over 95 per cent in the

normal strain (see Table 1). Thus the phenotype of *rfr* 10 is *rfr*⁺ *osp*⁻ (*osp*=oligosporogenous, that is, deficient in sporulation but producing spores at a frequency higher than the genetic reversion rate¹³).

Characterization of *rfr* 10

As shown in Table 1, mutant *rfr* 10 is resistant to rifampicin *in vivo*; growth is normal both in liquid medium and on plates containing rifampicin. On solid media favouring sporulation, *rfr* 10, like many mutants defective in sporulation, forms light-coloured, wet colonies¹⁴. *Rfr* 10 is not dependent on the presence of rifampicin for growth.

Extracts of logarithmically growing cells of *rfr* 10 were made by the method previously described³, which entails sonication, centrifugation at 100,000*g*, and precipitation of RNA polymerase from the supernatant with ammonium sulphate at 60 per cent saturation. As shown in Table 2, RNA polymerase from *rfr* 10 is resistant to rifampicin *in vitro*. An unexpected finding was that these extracts were poorly active with the synthetic template poly dAT, recalling the reduced ability of extracts of sporulating cells of *rfr* 11 to transcribe poly dAT (Table 2 and ref. 3).

Table 2. ACTIVITY OF RNA POLYMERASE EXTRACTED FROM *rfr* MUTANTS

Strain	Specific activity with ϕ DNA				Specific activity with poly dAT			
	Log-phase -rif	Log-phase +rif	Sporulating phase -rif	Sporulating phase +rif	Log-phase -rif	Log-phase +rif	Sporulating phase -rif	Sporulating phase +rif
3610	17.2	0.1	0.3	—	6.3	0.1	10.6	0.3
<i>rfr</i> 11	17.9	19.2	0.25	—	7.85	7.4	2.8	3.0
<i>rfr</i> 10	14.6	15.5	3.4	3.9	0.8	—	1.3	—
<i>rfr</i> 10 rev 8	15.7	5.2	0.86	0.50	2.0	1.4	2.2	0.84
<i>rfr</i> 3Y	13.8	11.0	8.7	7.3	4.4	4.3	0.7	0.7

For each strain, RNA polymerase was prepared by sonication, high-speed centrifugation, and precipitation with ammonium sulphate at 60 per cent saturation as previously described³ from cells harvested either in mid-log phase or 5 h after the end of logarithmic growth ("sporulating phase"). Medium 121B and the synchronized culture system of A. L. S. and Roscoe⁴ were used throughout. Enzymes were assayed as previously described³ with either phage ϕ DNA or poly dAT as template and, where applicable, in the presence of 0.4 μ g/ml. rifampicin or rifamycin. One unit of activity is that amount of enzyme that incorporates 1 nmole of ¹⁴C-AMP in 10 min. Specific activity is in units/mg protein.

RNA Polymerase from Cells of *rfr* 10 in Stationary Phase Cells transcribes ϕ DNA

The fact that *rfr* 10 does not sporulate with the same efficiency as wild-type *B. subtilis* suggested the possibility that RNA polymerase from *rfr* 10 might not undergo the same change in template specificity as the wild-type enzyme. To test this possibility, extracts were prepared from cells in mid-log phase and at several points in stationary phase, including times at which wild-type cells would completely exclude phage ϕ growth and produce an RNA polymerase completely inactive with phage ϕ DNA *in vitro*^{3,4}. Samples were also taken for measurement of phage burst size.

As shown in Fig. 1, RNA polymerase from *rfr* 10 was active with phage ϕ DNA at all times tested and the pattern of specific activity closely followed the pattern of phage burst size. Table 2 shows that polymerase activity with phage DNA as template was rifampicin-resistant in late stationary phase extracts as well as in log-phase extracts. A mixture of the log-phase extract with that of the last time-point (7.3 h) gave an activity equivalent to the sum of the individual activities of the two extracts. At all times tested, RNA polymerase from *rfr* 10 had low activity with the synthetic polymer poly dAT.

The finding that a mutation in RNA polymerase can block spore formation without affecting vegetative growth implies that the mutated component plays a specific part in sporulation. That the same mutation prevents the change in template specificity characteristic of sporulating wild-type cells suggests that it is this

Table 1. SPORE FORMATION AND RIFAMPICIN-RESISTANCE OF *rfr* MUTANTS

Strain	Growth in liquid		Colony formation Fraction of resistant cells	Spore formation Fraction of refractile heat-resistant cells	
	-rif	+rif		-rif	+rif
3610	+	—	10 ⁻⁶	0.90	1.0
<i>rfr</i> 11	+	+	0.78	0.95	1.0
<i>rfr</i> 10	+	+	1.0	0.05	0.08
<i>rfr</i> 10 rev 8	+	+	4 × 10 ⁻³	0.90	1.25
3Y	+	+	1.1	0.001	10 ⁻³

Each strain was transferred to medium 121B² (± 5 μ g/ml. rifampicin) after overnight growth in medium 121A². Growth was scored after 24 h of incubation at 37° C. For colony formation, cultures grown overnight in medium 121A were diluted and spread on plates of nutrient broth agar¹⁷ with or without 5 μ g/ml. rifampicin. Plates were incubated overnight at 30° C. Spore formation was tested in two ways. The culture grown for 24 h in medium 121B was examined for refractile spores by phase-contrast microscopy and an aliquot of the same culture was heated to 80° C for 10 min before being diluted and plated on nutrient broth agar; a control was plated before heating. The number of spores produced by strains *rfr* 11 or *rfr* 10 was not affected by the presence of 5 μ g/ml. rifampicin.

change itself that is decisive in determining ability to sporulate.

Rfr 10 Phenotype arises from Single Mutation

Three approaches served to confirm that the *rfr* 10 phenotype is the result of a single mutation: the spontaneous reversion frequency to the phenotype *rfr*⁺ *sp*⁺ was determined, the properties of the revertants were studied in detail, and additional mutants of the *rfr*⁺ *sp*⁻ class were isolated without mutagenesis.

Revertants of mutant *rfr* 10 were obtained by selecting for cells that had regained the ability to sporulate. A single colony of *rfr* 10 was suspended in sporulation medium 121B (see ref. 4) and shaken for 24 h at 37° C. The spores, a small proportion of the population, were isolated by washing the cells in distilled water and layering an aliquot of the washed cells on a linear gradient of 'Renografin 76' (Squibb; see ref. 15) constructed so that spores and vegetative cells would be separated on the

to rifampicin with the ability to sporulate. A representative of this class, *rfr* 10 *rev* 8, was chosen for further study.

Properties of *rfr* 10 *rev* 8

The revertant *rfr* 10 *rev* 8 was sensitive to rifampicin (and rifamycin) in both liquid and solid media (see Table 1). In the absence of the drug, this strain seemed to produce as many refractile, heat-resistant spores as the wild-type strain, but small differences in spore production would not be detectable.

Extracts of *rfr* 10 *rev* 8 were prepared as usual from log-phase and sporulating phase cells and tested for ability to transcribe phage ϕ e DNA and poly dAT and for sensitivity to rifampicin *in vitro*. Log-phase extracts were partially sensitive to rifampicin at the concentration used and had regained the ability to transcribe poly dAT (Table 2). Extracts of sporulating cells were also sensitive to rifampicin with the synthetic polymer as a

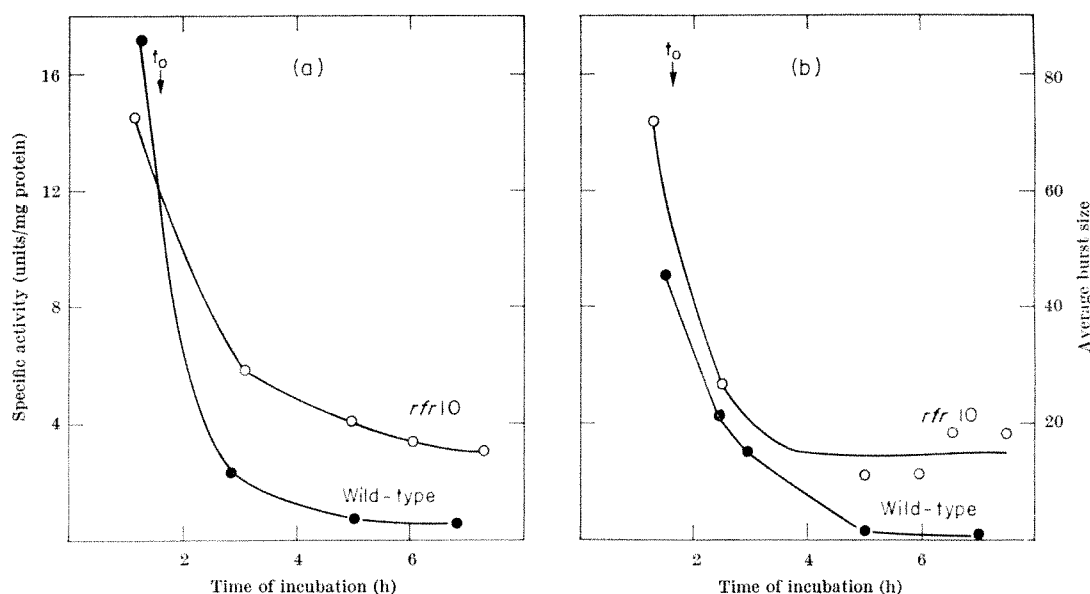


Fig. 1. Time course of change in template specificity in *B. subtilis* 3610 and mutant *rfr* 10. Cells were grown in 2 l. of medium 121B using the synchronized culture system of A. L. S. and Roscoe⁴. *a*, At the indicated times, 450 ml. samples were harvested, extracts were made as previously described³, and the activity of RNA polymerase was determined with phage ϕ e DNA or poly dAT as template as previously described³. Specific activity is defined in the legend to Table 2. *b*, At the indicated times, 0.9 ml. samples of each culture were infected with phage ϕ e at multiplicity less than 0.1 and average burst sizes were determined⁴. The end of logarithmic growth (t_0), the presumed starting point for sporulation, is shown by arrows.

basis of their respective densities. Of the single colonies derived from streaking the purified spores on nutrient agar plates, most gave the same small percentage of spores as the parent *rfr* 10 strain when regrown in medium 121B. These spores, which were resistant to plating on rifampicin-containing agar, were not genetic revertants, but rather a reflexion of the leakiness of the mutation.

A second class of spores gave rise to cultures that sporulated normally when regrown in medium 121B and were true revertants for the *sp* character. When tested for resistance to rifampicin, these strains showed the same sensitivity to the drug as wild-type *B. subtilis* 3610, indicating that the *sp* and *rfr* markers can revert simultaneously. The frequency of true revertants was 10^{-5} to 10^{-6} , a value consistent with reversion at a single mutated site. A similar frequency was found by searching on sporulation agar for dry, brown colonies¹⁴ derived from plating a 24 h culture of *rfr* 10 preheated to 80° C for 10 min. Some of the simultaneous revertants for both phenotypes did not completely regain sensitivity

template. More important, as a result of the reverting mutation, *rfr* 10 *rev* 8 lost the ability to transcribe ϕ e DNA during sporulation, a characteristic of wild-type cells. Fig. 2 compares the template activity of RNA polymerase from late stationary phase cells of wild-type, *rfr* 10, and *rfr* 10 *rev* 8. The revertant is altered in all of the properties that distinguish *rfr* 10 from the wild-type strain, strongly suggesting that these characteristics are the result of a single mutation.

Spontaneous *rfr*⁺ *sp*⁻ Mutants

Because *rfr* 10 had been isolated after ultraviolet mutagenesis, additional, spontaneous mutants of the *rfr*⁺ *sp*⁻ phenotype were sought. Single colonies of *B. subtilis* 3610 were grown overnight in medium 121A and spread without dilution on plates of LB agar (ref. 16; agar concentration, 2 per cent) or nutrient broth agar¹⁷ containing 5 μ g/ml. rifampicin or rifamycin (Lepetit). After overnight incubation at 30° C, a representative *rfr*⁺ colony from each clone was picked, purified by

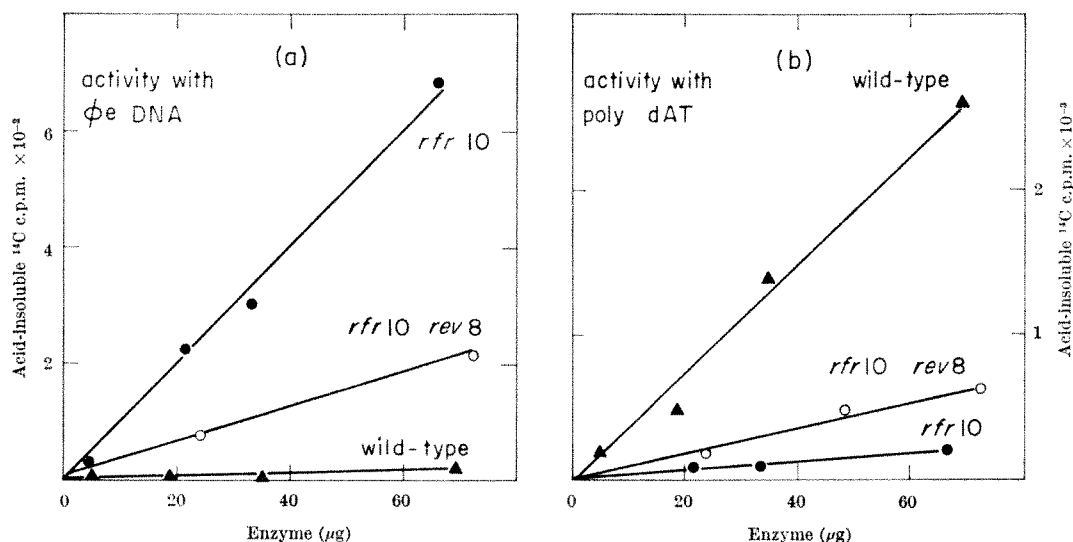


Fig. 2. Template activity of RNA polymerase from late stationary phase cells of 3610, *rfr* 10, and *rfr* 10 *rev* 8. RNA polymerase was prepared from cells of 3610, *rfr* 10, and *rfr* 10 *rev* 8 harvested 5 h after the end of logarithmic growth³. Each extract was assayed at varying protein concentrations with (a) ϕ DNA or (b) poly dAT as template as previously described³.

streaking on drug-containing agar, and tested for sporulation by growth in medium 121B. In three separate experiments, the frequency of the sporulation-deficient class among the *rfr* mutants was four of ten, one of three, and one of five. In all cases, the frequency of the *rfr* phenotype for individual clones was 10^{-7} to 10^{-8} . This result again indicates that the *rfr* *sp*⁻ phenotype can be the result of a single mutation.

One of these spontaneous mutants, termed *rfr* 3Y, was particularly interesting. It is only slightly leaky in spore production (see Table 1). During stationary phase, its RNA polymerase retains more than 50 per cent of the ability of the log-phase enzyme to transcribe phage ϕ DNA (Table 2); ability to transcribe poly dAT is lost specifically during stationary phase.

Rfr Mutants and Sporulation

The finding that some *rfr* mutants do not sporulate indicates that mutations in RNA polymerase can affect sporulation specifically, for these mutants are not altered in vegetative growth. This conclusion implies that RNA polymerase normally plays a part in differentiating between growth and sporulation, a role interfered with by this mutation.

Rfr mutations in *E. coli* are known to be localized in the core of RNA polymerase^{11,18}, that part of the enzyme which remains after selective removal of the σ factor⁷. Rabussay and Zillig¹⁹ have recently shown that the β protein component of the core enzyme is altered in at least one *rfr* mutant of *E. coli*. In the *B. subtilis* system we have shown that the core enzyme is sensitive to rifampicin (unpublished observations). In a following article⁸ we present evidence that sporulation core contains a 110,000 dalton subunit in place of one of the two 155,000 dalton β polypeptides of vegetative core and suggest that this alteration is responsible for the loss of ability of RNA polymerase to transcribe ϕ DNA. It is tempting to speculate that RNA polymerase in *rfr* 10 and *rfr* 3Y cells retains the ability to transcribe ϕ DNA during stationary phase because it is unable to undergo this alteration.

That a single mutation in RNA polymerase can prevent both sporulation and the change in template specificity demonstrates that these two phenomena are inter-related. The loss of vegetative template specificity may

be responsible for the turn-off of vegetative genes during sporulation; *rfr* 10 and *rfr* 3Y may fail to sporulate because they continue to express vegetative genes during stationary phase or because they fail to express sporulation genes. Consistent with this hypothesis is the finding that genes for ribosomal RNA are apparently shut off soon after the end of log-phase growth in wild-type cells but continue to be transcribed in cells of *rfr* 10 (unpublished work of C. Hussey, R. L. and A. L. S.). We plan to verify this hypothesis by testing whether vegetative polymerase, and not sporulation polymerase, will synthesize ribosomal RNA *in vitro* with *B. subtilis* DNA as a template.

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Structural Alteration of RNA Polymerase during Sporulation

by

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RNA polymerase from sporulating cells of *B. subtilis* has a subunit structure different from the vegetative enzyme. This alteration of RNA polymerase may be responsible for the turn-off of vegetative genes during spore formation.

THE kinds of enzyme activities and messenger RNA molecules found in sporulating cells of *Bacillus subtilis* are strikingly different from those found in vegetative cells¹⁻⁴. It is likely that these differences are brought about by the expression of new classes of genes during sporulation and the turn-off of certain vegetative genes, though little is known about the mechanism by which this change-over is controlled. Recently, Travers^{5,6} and Summers and Siegel⁷ have suggested that changes in the template specificity of RNA polymerase after phage infection are responsible for the expression of new phage genes and possibly the turn-off of host genes. In the case of coliphage T4, host RNA polymerase σ factor is replaced during infection by a new phage-induced σ factor, which directs the transcription of a different portion of the phage genome than does the σ factor of uninfected cells. We previously reported findings that suggested that a similar mechanism controls gene expression at the transcriptional level during bacterial sporulation⁸. Early in the process leading to sporulation, *B. subtilis* RNA polymerase changes dramatically in template specificity; it completely loses the ability to transcribe phage ϕ DNA, a finding consistent with the known inability to grow or produce phage specific antigens in sporulating cells. In the preceding article⁹, we provide evidence that this change is critical for sporulation because mutants that alter the RNA polymerase in such a way that it retains the ability to transcribe phage ϕ DNA also cause failure to sporulate. The loss of "vegetative" template specificity may be responsible for the turn-off of vegetative genes during spore formation.

Here we report that during sporulation the core of RNA polymerase undergoes an alteration which may be responsible for the loss of vegetative template specificity. We have reversibly fractionated vegetative RNA polymerase into a 57,000 molecular weight σ factor and a core enzyme by phosphocellulose chromatography. The core enzyme transcribes the synthetic template poly dAT but not ϕ DNA; transcription of the phage DNA is restored by addition of σ factor to the vegetative core. Phosphocellulose enzyme from sporulating cells contains an altered β subunit and does not transcribe ϕ DNA even after addition of σ factor from vegetative RNA polymerase. The failure of sporulation phosphocellulose enzyme to complement vegetative σ factor may be responsible for the loss of vegetative template specificity.

Transcription of ϕ DNA requires a σ Factor

RNA polymerase was purified from vegetative cells of *B. subtilis* strain 3610 by ammonium sulphate fractionation, DEAE-cellulose column chromatography, DNA-cellulose chromatography and zone centrifugation. After sedimentation through a glycerol gradient, RNA polymerase activity coincides with a protein peak; the enzyme is therefore highly purified. Purified enzyme transcribes both phage ϕ DNA and poly dAT (Table 1). The purified polymerase was then applied to a phosphocellulose column and eluted between 0.25 and 0.4 M KCl. This procedure is known to release a factor needed for the transcription

of phage T4 DNA from *E. coli* RNA polymerase¹². After phosphocellulose chromatography, the vegetative RNA polymerase fails to transcribe phage ϕ DNA, although it is even more active than the intact enzyme with poly dAT as a template (Table 1). Zone centrifugation showed that phosphocellulose enzyme has a sedimentation coefficient of 13.5 S compared with 15.4 S for RNA polymerase before the phosphocellulose step (Fig. 1).

These results suggest that phosphocellulose chromatography removes from vegetative polymerase a factor needed for the transcription of ϕ DNA, leaving a core enzyme which transcribes poly dAT. This factor can be found in the flow-through of the phosphocellulose column and will restore transcription of the phage DNA when added to vegetative core enzyme. Phosphocellulose flow-through was obtained from enzyme purified either through DEAE-cellulose or DNA-cellulose chromatography. Fig. 2 shows that the activity of RNA polymerase of the core fraction with ϕ DNA is stimulated several-fold by the addition of flow-through to vegetative core enzyme.

E. coli RNA polymerase can also transcribe ϕ DNA,

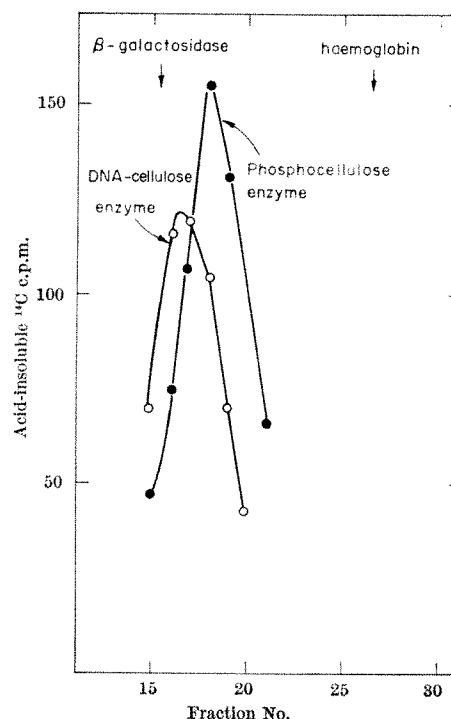


Fig. 1. Zone centrifugation of RNA polymerase. Samples containing, in 0.5 ml., 50 μ g of vegetative DNA-cellulose enzyme and 50 μ g of vegetative core enzyme were layered on parallel 12 ml. linear gradients of 10–30 per cent glycerol in buffer containing 0.01 M Tris-HCl (pH 7.9), 0.01 M MgCl₂, 0.0001 M EDTA, 0.0001 M dithiothreitol and 0.15 M KCl. Following centrifugation for 15 h at 40,000 r.p.m. in an International 'SB 283' rotor at 3° C, fractions (0.4 ml.) were collected and 0.025 ml. of each fraction was assayed as previously described⁸. Two molecular weight markers, haemoglobin (4.6 S) and β -galactosidase (16 S), were centrifuged on a parallel gradient.

Table 1. SPECIFIC ACTIVITY OF VEGETATIVE AND SPORULATION RNA POLYMERASE

Enzyme	Specific activity (units/mg protein)	
	ϕ e DNA	Poly dAT
Vegetative	198	77
Vegetative core	18	320
Sporulation	5	87
Sporulation core	6	250
Vegetative (2 μ g) and sporulation (9 μ g)	199	—

Vegetative cells were obtained by growing *B. subtilis* 3610 in medium 121B¹⁴ and harvesting during mid-log phase. Cells were disrupted by sonication and vegetative RNA polymerase purified by high speed centrifugation, ammonium sulphate fractionation and DEAE-cellulose column chromatography as previously described⁴. After the ammonium sulphate step, a volume equal to 5 per cent of the volume of the extract of a phenylmethane-sulphonyl fluoride solution (6 mg/ml of ethanol) was added to inhibit proteases. The vegetative enzyme was further purified by calf-thymus DNA-cellulose column chromatography using stepwise elution with 0.15–0.6 M KCl according to the method of Alberts *et al.*¹¹. Finally, the enzyme was sedimented through a 10–30 per cent glycerol gradient containing 0.15 M KCl. Vegetative core enzyme was obtained by applying glycerol gradient enzyme to a phosphocellulose column and eluting stepwise between 0.25 and 0.4 M KCl¹². Sporulating cells were also grown in 121B¹⁰ medium and harvested 6 h after the end of logarithmic growth. Cells were disrupted by sonication and sporulation enzyme purified by high speed centrifugation and ammonium sulphate fractionation⁴. The enzyme was next applied to a DEAE-cellulose column and eluted with a 0.1 to 0.3 M KCl gradient. The enzyme was further purified by glycerol density centrifugation. Sporulation core enzyme was obtained by phosphocellulose chromatography of the DEAE-cellulose enzyme as described in the legend to Fig. 5. Enzymes were assayed with either ϕ e DNA or poly dAT as previously described⁴. One unit of specific activity is that amount of enzyme which incorporated 1 nmole of ¹⁴C-AMP in 10 min.

and it seemed possible that *E. coli* σ factor would enable *B. subtilis* core enzyme to transcribe the phage DNA. Fig. 2 shows that, in fact, *E. coli* σ factor greatly stimulates vegetative *B. subtilis* core enzyme with ϕ e DNA as a template. We conclude that vegetative *B. subtilis* RNA polymerase consists of a core enzyme plus a σ factor necessary for the transcription of ϕ e DNA. The factor is sufficiently similar to the *E. coli* σ factor to allow the latter to complement *B. subtilis* core enzyme. Kerjan and Szulmajster have also released a factor from *B. subtilis* polymerase by phosphocellulose chromatography¹³.

Polypeptide Composition of Vegetative RNA Polymerase

The subunit structure of vegetative RNA polymerase was analysed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis, which separates polypeptides on the basis of molecular weight. Fig. 3 shows the gel pattern for vegetative enzyme and vegetative core enzyme. Vegetative enzyme preparations contain polypeptides of 155,000, 120,000, 57,000 and 45,000 daltons. In gels of phosphocellulose enzyme, the 57,000 dalton

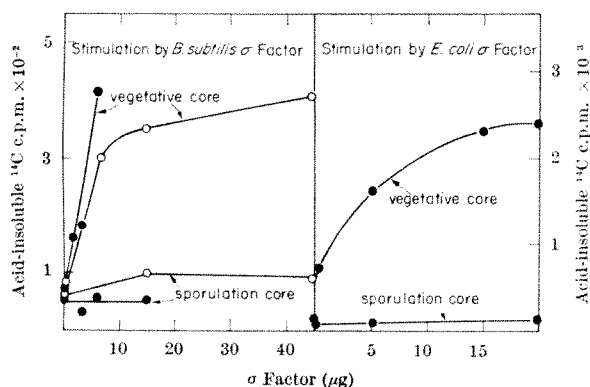


Fig. 2. Stimulation of core enzyme by σ factor. *B. subtilis* σ factor was obtained by phosphocellulose chromatography of vegetative enzyme purified either only through the DEAE-cellulose step (○) or through the DNA-cellulose step (●). Varying amounts of flow-through from the phosphocellulose chromatography were added to 3 μ g of vegetative core enzyme or 4 μ g of sporulation core enzyme and assayed as previously described with ϕ e DNA as a template⁴. In the case of σ factor derived from DEAE-cellulose enzyme, a background value of 7 c.p.m./ μ g of flow-through has been subtracted because of transcription of ϕ e DNA in the absence of added core enzyme. A value of 39 c.p.m./ μ g of flow-through has been subtracted in the case of σ factor derived from DNA-cellulose enzyme. This background incorporation is the result of small amounts of core enzyme present in the phosphocellulose flow-through. *E. coli* σ factor was the gift of R. R. Burgess and was assayed in the presence of 3 μ g of vegetative core or 4 μ g of sporulation core. A background value of 11 c.p.m./ μ g of σ factor was subtracted.

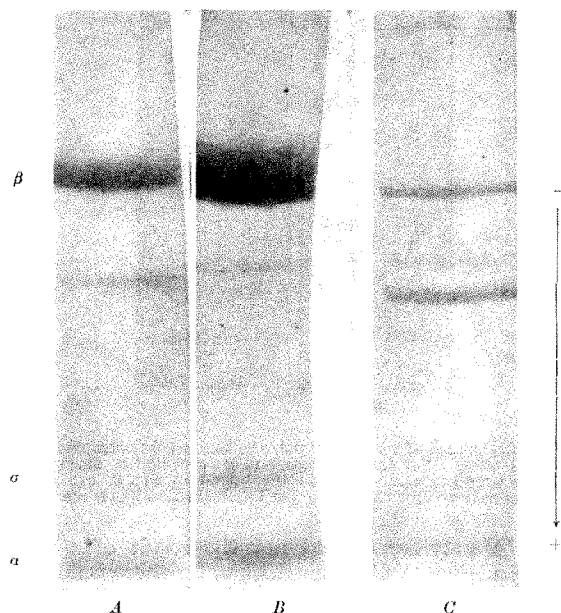


Fig. 3. SDS polyacrylamide gel electrophoresis patterns of vegetative and sporulation RNA polymerase. From left to right: vegetative core (A); vegetative glycerol gradient enzyme (B); sporulation core (C). Samples were carboxymethylated according to the method of Pringle¹⁴ before electrophoresis. Carboxymethylation destroys proteases which may be present in trace amounts and are resistant to SDS and attack other proteins during denaturation in SDS¹⁴. Gels containing 0.1 per cent SDS and 5 per cent acrylamide were run for 3 h at 8 mA per gel according to the procedure of Weber and Osborn¹⁵. The gels were then stained with Coomassie brilliant blue and destained electrophoretically according to Weber and Osborn¹⁵.

subunit is the only one missing. It is found, instead, in gels of the flow-through of phosphocellulose chromatography. The 57,000 dalton subunit therefore seems to be the σ factor of vegetative RNA polymerase. Avila *et al.*¹⁵ have reported a similar molecular weight for the *B. subtilis* σ factor. In view of our finding that both *B. subtilis* and *E. coli* factors complement *B. subtilis* core enzyme, it is interesting to note that the factor of *B. subtilis* is considerably smaller than the *E. coli* factor (95,000 daltons¹²).

Electrophoresis for extended times resolves the 155,000 dalton band into two polypeptides (Fig. 4) which probably correspond to the β and β' polypeptides of *E. coli* RNA polymerase (about 160,000 daltons¹⁶). The 45,000 dalton band presumably represents the α polypeptide (40,000 daltons in *E. coli*¹⁶). The molar ratio of the 45,000 and 155,000 dalton bands calculated from densitometer tracings is 1:1.1, which is in good agreement with the $\alpha_2\beta\beta'$ structure of *E. coli* RNA polymerase¹⁶. The 120,000 dalton polypeptide is probably a contaminating protein; it is present in variable amounts in different preparations and is absent in at least some preparations that are active with both poly dAT and ϕ e DNA. It seems therefore that vegetative RNA polymerase from *B. subtilis* has a subunit composition similar to that of the *E. coli* enzyme consisting of α , β , β' and σ polypeptides.

Vegetative σ Factor does not complement Sporulation Core Enzyme

Sporulation RNA polymerase was partially purified by ammonium sulphate fractionation, DEAE-cellulose column chromatography and zone centrifugation. Polymerase activity was found to coincide with a protein shoulder after sedimentation through a glycerol density gradient. Chromatography on calf thymus DNA-cellulose was not used because it was found that sporulation polymerase binds poorly to such a column. Table 1 shows that the sporulation enzyme fails to transcribe ϕ e DNA even though it is active with poly dAT as a template. Failure to transcribe the phage DNA is an inherent property of sporula-

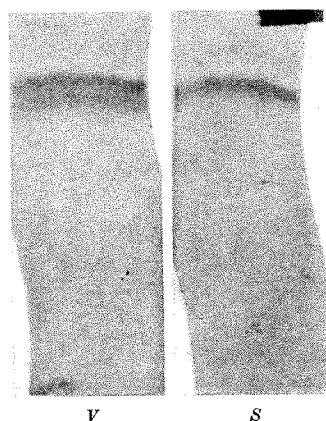


Fig. 4. Separation of β and β' by polyacrylamide gel electrophoresis for extended time. Vegetative enzyme (V); sporulation enzyme (S). SDS gels were run as described in the legend to Fig. 3, except that electrophoresis was for 8 h. Only the 155,000 dalton bands are shown.

tion RNA polymerase and not the result of a specific antagonist of ϕ e DNA transcription, for there is no inhibition of transcription of ϕ e DNA when sporulation enzyme is added to vegetative enzyme (Table 1).

Sporulation enzyme purified through the DEAE-cellulose step was applied to a phosphocellulose column. The flow-through of the column did not stimulate transcription of ϕ e DNA by vegetative core enzyme. Thus if sporulation enzyme contained vegetative σ factor, this factor is not released by phosphocellulose chromatography. Sporulation enzyme was eluted from the column with a linear KCl gradient. Fig. 5 shows that enzyme activity corresponded to a protein peak, so that the enzyme is highly purified. We shall provisionally call the activity purified by phosphocellulose sporulation "core" enzyme. Fig. 2 shows that sporulation core enzyme does not transcribe phage ϕ e DNA even after addition of σ factor derived from either vegetative *B. subtilis* polymerase or from *E. coli* polymerase.

Sporulation RNA Polymerase contains an Altered β Polypeptide

Sporulation core enzyme contains polypeptides of 155,000 and 45,000 daltons and small amounts on a molar basis of a 120,000 molecular weight species (Fig. 3). These polypeptides are similar in molecular weight to corresponding polypeptides of vegetative core enzyme. In addition, there is in the sporulation core enzyme a polypeptide of 110,000 molecular weight. The following evidence indicates that this new polypeptide is present in place of one of the β polypeptides. (a) The 155,000 dalton band of sporulation enzyme does not separate into two polypeptides after extended electrophoresis as does the vegetative β band (Fig. 4). (b) The molar ratio of the 45,000 dalton band to the 110,000 and the 155,000 dalton polypeptides is 1:0.54:0.47. Thus there is approximately only half as much of the 155,000 subunit species in the sporulation enzyme as in the vegetative enzyme. These findings suggest that one of the 155,000 dalton polypeptides is missing and is replaced in sporulation polymerase by a new subunit of 110,000 molecular weight. Sporulation core also contains small amounts of material of about 60,000 daltons. We cannot therefore conclude that sporulation core enzyme is entirely free of either vegetative or sporulation σ factors.

To test the possibility that the structural alteration of RNA polymerase takes place *in vitro* during purification rather than *in vivo* during sporulation, 6 g of vegetative cells was mixed with 18 g of sporulating cells. The mixture was sonicated and RNA polymerase was partially purified by ammonium sulphate fractionation. Next, vegetative enzyme was separated from sporulation enzyme by taking advantage of the observation mentioned

here that sporulation RNA polymerase binds poorly to a calf-thymus DNA-cellulose column. Vegetative RNA polymerase, eluted from the DNA-cellulose column and sedimented through a glycerol density gradient, was still active with ϕ e DNA as a template, and on SDS gel analysis revealed the same subunit structure as vegetative enzyme. This result argues that structural alteration of sporulation RNA polymerase takes place *in vivo* and is not the result of proteolysis during purification.

Conclusions

In an earlier publication⁸ we showed that RNA polymerase loses the ability to transcribe ϕ e DNA early during sporulation. Here we show that the vegetative enzyme depends on a σ factor of 57,000 molecular weight of the transcription of ϕ e DNA. Sporulation polymerase that has been purified by phosphocellulose chromatography will not transcribe ϕ e DNA even when the σ factor from vegetative enzyme is supplied. One possibility is that sporulation polymerase contains a sporulation σ factor that is not released by phosphocellulose chromatography and that this sporulation σ factor prevents complementation by vegetative σ factor. An attractive possibility, not incompatible with the above hypothesis, is that RNA polymerase core is changed in such a way that it cannot complement vegetative σ factor. This might account for the loss of vegetative template specificity during sporulation. It should be noted, however, that this need not be the only mechanism, for inactivation of σ early during sporulation would also prevent the transcription of ϕ e DNA.

Analysis of subunit structure by SDS gel electrophoresis has revealed a difference between vegetative and sporulation RNA polymerase cores. Vegetative RNA polymerase contains two β polypeptides of about 155,000 molecular weight. In the sporulation RNA polymerase one of these polypeptides is only 110,000 daltons. This feature of the sporulation core enzyme may be the modification responsible for its failure to complement vegetative RNA polymerase σ factor. It is interesting to note that after phage T4

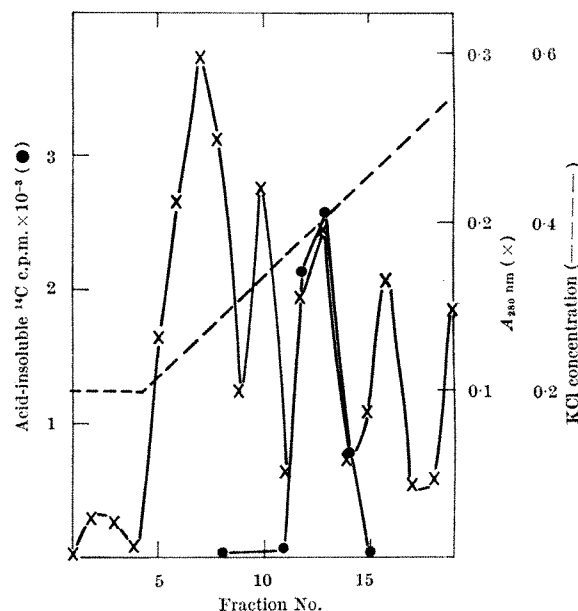


Fig. 5. Phosphocellulose chromatography of sporulation RNA polymerase. Phosphocellulose (Whatman 'P11', 7.4 mequiv/g) was washed with acid and base and titrated to pH 7.9 according to the method of Burgess *et al.*¹². This material was used for a 30 ml. column and equilibrated extensively with buffer containing 0.05 M Tris-HCl (pH 7.9 at 25°C), 0.1 M KCl, 0.0001 M EDTA, 0.0001 M dithiothreitol and 20 per cent glycerol. A sample containing 78 mg of sporulation RNA polymerase purified through the DEAE-cellulose step in 24 ml. of the above buffer containing 0.12 M KCl was applied to the column over the course of 1 h. The column was washed with buffer containing 0.15 M KCl and then 0.25 M KCl and then eluted with a 100 ml. gradient from 0.2 M to 0.8 M KCl. 4 ml. fractions were collected.

infection of *E. coli*, both the α polypeptide¹⁷ and the β' polypeptide (personal communication of A. A. Travers) have altered electrophoretic properties and that *E. coli* σ factor does not stimulate this core polymerase from infected cells to the same extent that it stimulates core enzyme from uninfected cells (personal communication of C. Goff and A. A. Travers).

The 110,000 dalton polypeptide of sporulation RNA polymerase may be derived from one of the β polypeptides by specific proteolytic cleavage. Sadoff and Celikkol¹⁸ have shown that fructose-1,6-diphosphate aldolase is modified by such a proteolytic step during sporulation of *B. cereus*. It is not yet excluded that the 110,000 dalton polypeptide is synthesized specifically during sporulation and replaces one of the β polypeptides. In phage T4 infection, the alteration of the α polypeptide is the result of modification of the α subunit of uninfected cells rather than replacement by a new polypeptide (personal communication of C. Goff and K. Weber).

The preceding article describes a class of rifampicin-resistant mutants of *B. subtilis* which, as a result of a single mutational event, fail to sporulate and retain the ability to transcribe phage ϕ DNA. These mutants provide direct evidence that the change in the template specificity of RNA polymerase is essential for sporulation. Our present finding of an alteration of a β polypeptide of RNA polymerase during sporulation suggests an explanation for the inability of the polymerase of the rifampicin-resistant mutants to undergo change in template specificity. Rifampicin is a specific inhibitor of RNA polymerase core and RNA polymerase from at least one *E. coli* rifampicin-resistant mutant contains an altered β polypeptide¹⁹. Mutation to rifampicin resistance in *B. subtilis* may modify a β polypeptide in such a way as to make it resistant to specific proteolytic cleavage. Thus the non-sporulating, rifampicin-resistant mutants of *B. subtilis* may retain the ability to transcribe ϕ DNA during stationary phase because the RNA polymerase core enzyme remains unmodified.

There is reason to believe that at least some vegetative genes are turned off during sporulation. Various enzyme activities and species of messenger RNA found in vegetative cells are not present in sporulating cells^{1,3,4}. In

addition, the synthesis of ribosomal RNA is arrested during sporulation (unpublished results of C. Hussey, R. L. and A. L. S.). Assuming that the vegetative RNA polymerase σ factor which we have described directs the transcription of these vegetative genes, the molecular event responsible for the turn-off of vegetative genes might be a structural alteration of RNA polymerase. Many new genes are almost certainly transcribed during sporulation. This transcription may be directed by specific sporulation factors. We are now searching for such factors.

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Initiation of Haemoglobin Synthesis by Methionyl-tRNA

by

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The synthesis of haemoglobin in a cell free system containing yeast tRNA is initiated by a specific methionine tRNA. Another methionine tRNA responds to internal codons in the messenger. The initiating methionine is removed from the nascent protein to leave valine as the terminal amino-acid, but cannot be removed when its amino group is blocked as in formylmethionine.

BACTERIA contain two major species of methionine tRNA, tRNA^{Met} and tRNA^{Met}_f (refs. 1, 2). The latter can be enzymatically formylated to fMet-tRNA^{Met}_f (ref. 3) and is used to initiate protein synthesis. tRNA^{Met} inserts its methionine only at internal positions of a polypeptide chain. The presence of fMet-tRNA^{Met}_f in mitochondria^{4,5} and in chloroplasts⁶ suggests that this tRNA is involved in the initiation of protein synthesis in these organelles

too. By contrast, the cytoplasm of eukaryotic cells does not contain a formylated methionyl-tRNA^{Met}_f.

Two species of methionine tRNA are present, however, in the cytoplasm of several eukaryotic cells, including guinea-pig liver⁷, rat liver⁸, yeast^{9,10} and wheat germ¹¹. When added to a bacterial cell-free protein synthesizing system, one of these, tRNA^{Met}_M, behaves similarly to *Escherichia coli* tRNA^{Met} and inserts methionine into the internal position of a polypeptide chain. The other, tRNA^{Met}_F, is not formylated *in vivo*, but can be formylated *in vitro* by *E. coli* transformylase. The yeast fMet-tRNA^{Met}_F thus formed initiates protein synthesis in extracts of *E. coli* with the same specific requirements and conditions as does *E. coli* fMet-tRNA^{Met}_f (refs. 9, 10).

Abbreviations used: tRNA^{Met}_f, the bacterial methionine tRNA which is formylated *in vivo*; tRNA^{Met}_M, the bacterial methionine tRNA which is not formylated *in vivo*; tRNA^{Met}_F, the yeast methionine tRNA which is not formylated *in vivo*, but which can be formylated by bacterial transformylase; tRNA^{Met}_M, the yeast methionine tRNA which is not formylated *in vitro* or *in vivo*. (The latter two designations do not represent definitive usage.—Editor.)

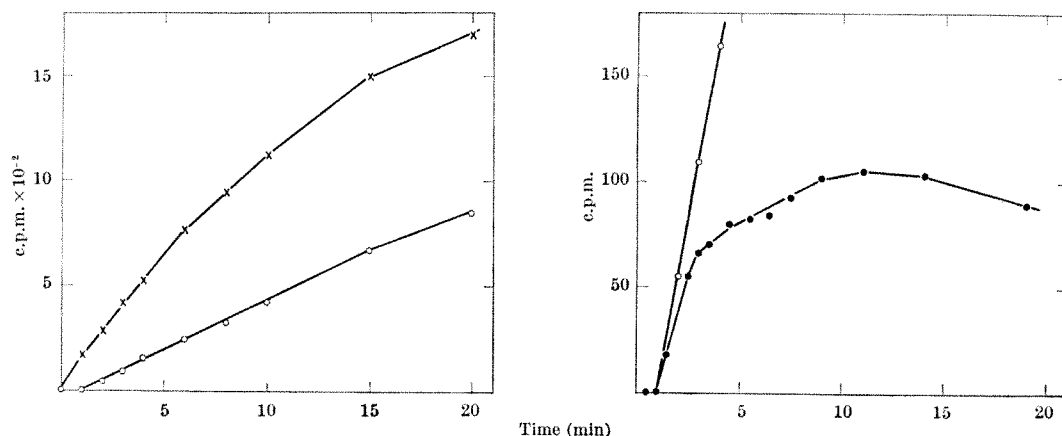


Fig. 1. Kinetics of amino-acid incorporation into protein. Data for formyl- ^{35}S -Met-tRNA $^{\text{MetF}}$ are plotted twice, on different scales, to show the relationships with the other two Met-tRNAs. O, Formyl- ^{35}S -Met-tRNA $^{\text{MetF}}$; \bullet , ^{35}S -Met-tRNA $^{\text{MetM}}$; \times , ^{35}S -Met-tRNA $^{\text{MetE}}$. All reactions contained (per ml.) 0.62 ml. reticulocyte lysate, 0.92 μmoles ATP, 0.19 μmoles GTP, 50 μmoles KCl, 5 μmoles mercaptoethanol, 11 μmoles creatine phosphate, 1 mg creatine phosphokinase, 25 μmoles HEPES (pH 7.0), 3.1 μmoles magnesium acetate, 34 μg haemin, 0.1 μmoles of all twenty amino-acids (non-radioactive), except non-radioactive methionine (1.2 μmoles). Where indicated, reactions contained (per ml.) 8×10^5 c.p.m. formyl- ^{35}S -methionyl-tRNA $^{\text{MetF}}$ (48,000 c.p.m./ μg tRNA; specific activity of ^{35}S -methionine, 5,000 mCi/mmol; 20 per cent counting efficiency), 5×10^5 c.p.m. ^{35}S -Met-tRNA $^{\text{MetM}}$ (30,000 c.p.m./ μg) or 8×10^5 c.p.m. ^{35}S -Met-tRNA $^{\text{MetE}}$ (about 4,000 c.p.m./ μg). Incubation was at 25°C ; at indicated times 15 μl . aliquots were taken into 0.5 ml. cold water. KOH was added to 0.1 M; after incubation at 37°C for 10 min, trichloroacetic acid (TCA) was added to 5 per cent. The precipitate was collected on fibreglass filters, washed with 5 per cent TCA, dried, and counted on an end window counter at about 20 per cent efficiency. Methionine transfer RNA, tRNA $^{\text{MetF}}$ and tRNA $^{\text{MetM}}$ were purified from yeast as described previously¹⁰; the former was at least 90 per cent pure, the latter 10 per cent. Neither preparation was contaminated with the other methionine tRNA species. Charging and formylation by the *E. coli* enzymes was also described previously¹⁰. To prepare the reticulocyte extracts¹⁸, New Zealand white rabbits, weighing 5 to 7 lb., were made anaemic by subcutaneous injection of 2.5 per cent phenylhydrazine solution according to the following schedule: 1.0 ml. on day 1, 0.8 ml. on day 2, 0.6 ml. on day 3, 0.8 ml. on day 4, 1.0 ml. on day 5. On the seventh day about 30 ml. of blood taken from the ear was collected into 30 ml. of buffer A (140 mM NaCl, 1.5 mM magnesium acetate, 5 mM KCl, 0.001 per cent heparin). After centrifugation, the reticulocytes were washed by centrifugation three times in buffer A without heparin. The packed reticulocytes were lysed by addition of 1 volume cold water; after centrifugation at 15,000 r.p.m. for 20 min in the 'Sorvall SS34' rotor, the lysate was stored frozen in small aliquots at -80°C .

Furthermore, yeast Met-tRNA $^{\text{MetF}}$ and *E. coli* Met-tRNA $^{\text{MetE}}$ are unable to insert methionine into the internal positions of a polypeptide in either *E. coli*¹² or in yeast (unpublished results of D. H., U. L. R. and H. F. L.) protein synthesizing systems.

The analogous properties of the yeast tRNA $^{\text{MetF}}$ and *E. coli* tRNA $^{\text{MetE}}$ suggest that this species might initiate the synthesis of eukaryotic proteins. We show now that haemoglobin synthesis in a cell-free system is initiated by Met-tRNA $^{\text{MetF}}$ and that the amino terminal methionine is enzymatically removed from the nascent product. If the methionine amino group is blocked by a formyl (or acetyl) group, the amino terminal methionine is not removed. By contrast, tRNA $^{\text{MetM}}$ donates its methionine only into the internal positions of the α and β chains.

Our results confirm the fundamental finding of Wilson and Dintzis¹³ that nascent α -haemoglobin polypeptides in intact reticulocytes contain methionine at their amino termini, and that the methionine residue is subsequently removed. Using synthetic polyribonucleotides as messenger and ribosomes from rabbit reticulocytes and ascites tumour cells, respectively, Gupta *et al.*¹⁴ and Smith, Marcker and Brown^{15,16} showed that methionine incorporated from Met-tRNA $^{\text{MetM}}$ (designated Met-tRNA $^{\text{M}}$ in refs. 15, 16) is found exclusively in internal positions of polypeptide chains, while that from Met-tRNA $^{\text{MetF}}$ (Met-tRNA $^{\text{F}}$ in refs. 15, 16) is found only amino terminal.

In cell-free extracts of rabbit reticulocytes more than 90 per cent of the protein produced is haemoglobin and the system initiates synthesis of new polypeptides^{19,20}. Fig. 1 shows that ^{35}S -methionine from yeast ^{35}S -Met-tRNA $^{\text{MetM}}$ is incorporated very efficiently into protein, whereas ^{35}S -Met-tRNA $^{\text{MetF}}$ seems to be poorly incorporated. Two other groups have made similar observations^{14,21}. When the methionine on tRNA $^{\text{MetF}}$ is formylated (fMet-tRNA $^{\text{MetF}}$), however, methionine radioactivity

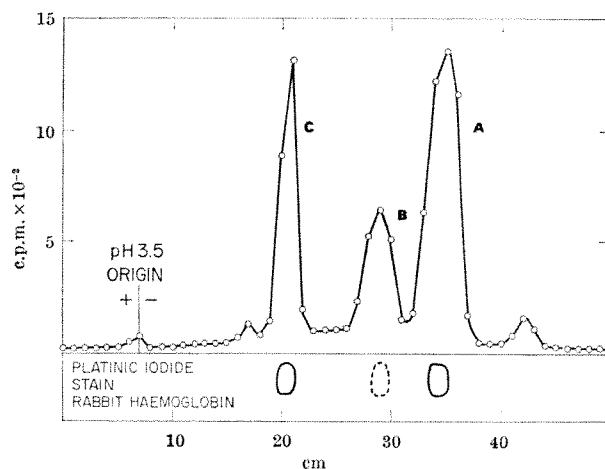


Fig. 2. Analysis of ^{35}S -Met-tRNA $^{\text{MetM}}$ product. A 0.1 ml. reaction mixture, containing ^{35}S -Met-tRNA $^{\text{MetM}}$ (Fig. 1), was incubated at 37°C for 10 min (11,400 c.p.m. was incorporated). Pancreatic ribonuclease (to 100 $\mu\text{g}/\text{ml}$) and EDTA (to 0.03 M) were added and incubation continued for 10 min. The sample was precipitated with trichloroacetic acid, centrifuged, and the precipitate washed by centrifugation three times with 5 per cent TCA. A small amount of water was added and the precipitate lyophilized until the solution was no longer acid. The precipitate was resuspended at 10 mg/ml. in 1 per cent NH_4HCO_3 containing trypsin (TPCK, Worthington) at 0.2 mg/ml. After incubation at 37°C for 4 h, the material was freeze-dried, and subjected to paper ionophoresis at pH 3.5 for 3 h at 45 V/cm. 1 cm strips were cut out and radioactivity was counted in a scintillation counter using a toluene-based scintillator; the strips were then washed in fresh toluene, dried, and stained for methionine as described in ref. 29. The spot indicated by the dotted line (29 cm) was considerably fainter than the other two. Free methionine would be at 12 cm.

Table 1. EFFECT OF ANTIBIOTICS ON PROTEIN SYNTHESIS

Addition	Formyl- ^{35}S -methionyl-tRNA $^{\text{MetF}}$	^{35}S -Methionyl-tRNA $^{\text{MetM}}$	^{35}S -Methionyl-tRNA $^{\text{MetE}}$
None	1.00 (1,466 c.p.m.)	1.00 (136 c.p.m.)	1.00 (4,017 c.p.m.)
Chloramphenicol (100 $\mu\text{g}/\text{ml}$.)	0.98	1.16	0.90
Cycloheximide (200 $\mu\text{g}/\text{ml}$.)	0.00	0.01	0.00
Aurintricarboxylate (10^{-4} M)	0.01	0.02	0.31

Reactions are described in the legend to Fig. 1; 15 μl . aliquots were counted after 10 min incubation at 37°C ; zero time backgrounds (8–12 c.p.m.) have been subtracted.

is transferred into protein almost as efficiently as from Met-tRNA^{Met}. Because proteins are synthesized from the amino terminus, incorporation of a formylmethionyl residue must represent initiation of new polypeptide chains.

Table 1 presents evidence that protein synthesis is occurring solely on cytoplasmic ribosomes. Incorporation by all three ³⁵S-Met-tRNAs is completely blocked by cycloheximide, an antibiotic which specifically inhibits synthesis by cytoplasmic (80S) ribosomes, but is without effect on bacterial and mitochondrial ribosomes. Aurintricarboxylate (ATA), a specific inhibitor of polypeptide chain initiation³⁵, completely blocks incorporation from ³⁵S-Met-tRNA^{Met} and its formylated derivative.

Incorporation by Met-tRNA^{Met}

Fig. 2 shows that tRNA^{Met} transfers methionine exclusively into internal positions of both the α and β chains. Protein synthesized *in vitro* and labelled with ³⁵S-Met-tRNA^{Met} was digested with trypsin and analysed by paper ionophoresis at pH 3.5 (Fig. 2). The three radioactive peptides observed had mobilities identical to the three methionine-containing peptides from authentic rabbit haemoglobin. Similar results (unpublished) were obtained by ionophoresis at pH 4.5. Peptides A and C are most likely the single methionine-containing tryptic peptides from the α and β chain, respectively^{22,23}, whereas B is most likely a derivative of C resulting from chymotrypsin impurities in the trypsin.

Incorporation by fMet-tRNA^{Met}

Several experiments show that formylmethionine residues from fMet-tRNA^{Met} are incorporated into the amino

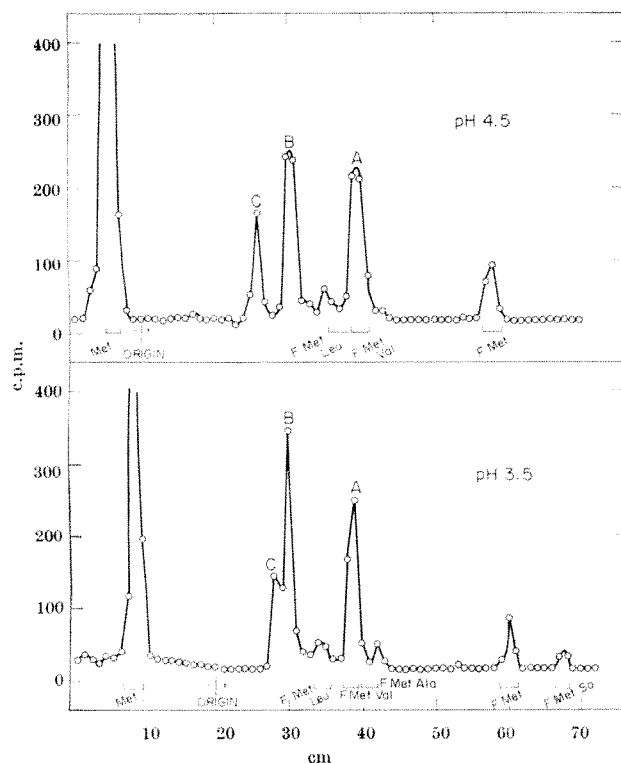


Fig. 3. Pronase digestion of formyl-³⁵S-methionyl product. Protein synthesized *in vitro* (10 min at 37°C, Fig. 1) containing formyl ³⁵S-Met-tRNA^{Met} was isolated as in Fig. 2, and digested with pronase³⁰ (Calbiochem B grade, 0.2 mg/ml.). A small amount of formyl-methionyl-valine was added and the product was analysed by paper ionophoresis at pH 3.5 and 4.5 fMet-Val and other markers were detected as in Fig. 2. The large peak of free methionine (1,100 c.p.m.) results from enzymic deformation of formylmethionine peptides by the pronase preparation. In the same conditions digestion of the bacteriophage f2 coat protein and RNA polymerase, synthesized *in vitro* and containing an amino terminal formyl-³⁵S-methionine residue³⁰, also yielded a large amount of radioactive methionine in addition to the formyl-³⁵S-methionyl amino-acids³⁰.

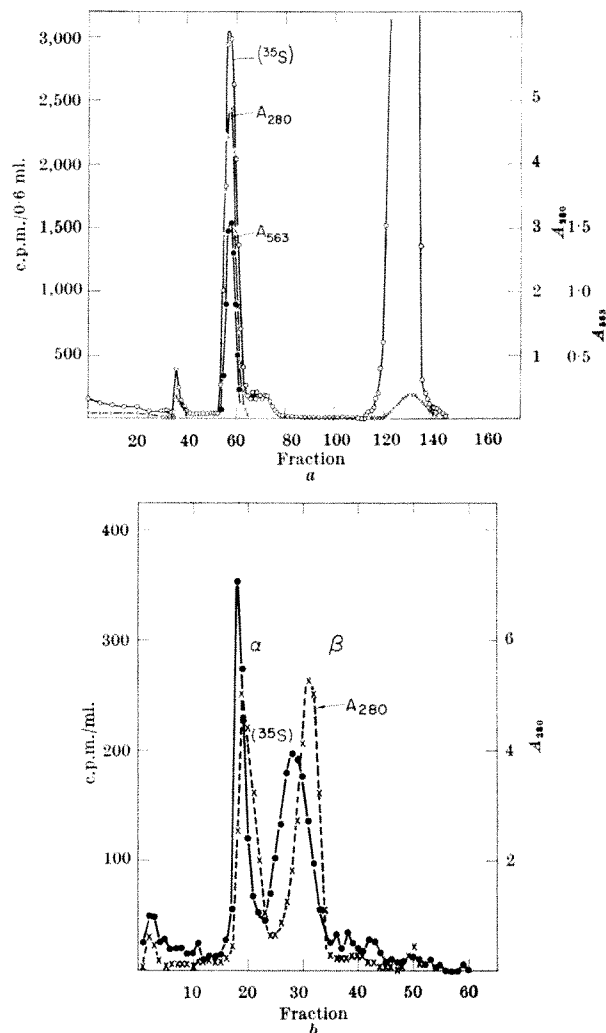


Fig. 4. Chromatography of formyl-³⁵S-methionyl reaction product. *a*, Sephadex G-75: ○, ³⁵S; ×, A₂₈₀ nm; ●, A₅₆₃ nm. *b*, Isolated globin chains on CM-cellulose: ●, ³⁵S; ×, A₂₈₀ nm. In (*a*), a 2.0 ml. *in vitro* reaction labelled with formyl-³⁵S-methionyl-tRNA^{Met} (Fig. 1; 20 min incubation at 25°C; total of about 220,000 c.p.m. protein) was treated with ribonuclease (Fig. 2), then chromatographed on a column (8.4 × 147 cm) of Sephadex G-75 equilibrated with 0.1 M NaCl-0.01 M Tris (pH 7.6)-0.001 M KCN. The same buffer was used for elution and 10 ml. fractions were collected. Haemoglobin, as measured by the haem absorbance at 563 nm, elutes in fractions 53-60; the large peak of radioactivity eluting between fractions 115 and 140 is low molecular weight material derived from the added tRNA; totally excluded material (dextran blue test) elutes at fraction 35. In (*b*), a 0.1 ml. reaction containing formyl-³⁵S-methionyl-tRNA^{Met} (Fig. 1) was stopped by chilling, and globin was prepared by precipitation with acid acetone (ref. 31); 50 mg purified rabbit globin was added, and the mixture was chromatographed on a column (0.9 × 23 cm) of CM-cellulose (Whatman CM32). Elution was performed as in ref. 32, except that all buffers contained 0.001 M 2-mercaptoethanol. 6 ml. fractions were collected; 1 ml. fractions were mixed with a dioxane-based scintillation fluid and counted in the scintillation counter.

terminus of haemoglobin. First, protein synthesized *in vitro* and labelled with N-formyl ³⁵S-methionyl-tRNA^{Met} was digested with pronase, and the products were analysed by paper ionophoresis at pH 3.5 and 4.5 (Fig. 3). Peak A was inseparable from the marker formylmethionyl-valine. Except formylmethionyl-asparagine and formylmethionyl-glutamine, which were not tested, all other N-formylmethionyl amino-acids were separable from peak A and formylmethionyl-valine at at least one pH (unpublished results of H. F. L.). Peptides B and C migrated more slowly than N-formylmethionyl-leucine, the slowest moving formylmethionyl dipeptide, and are therefore probably tri or tetrapeptides. Because valine is at the amino terminus of both the α and β chains of rabbit haemoglobin, these results suggest that at least one of the synthesized polypeptides is a haemoglobin chain beginning with the sequence fMet-Val. . .

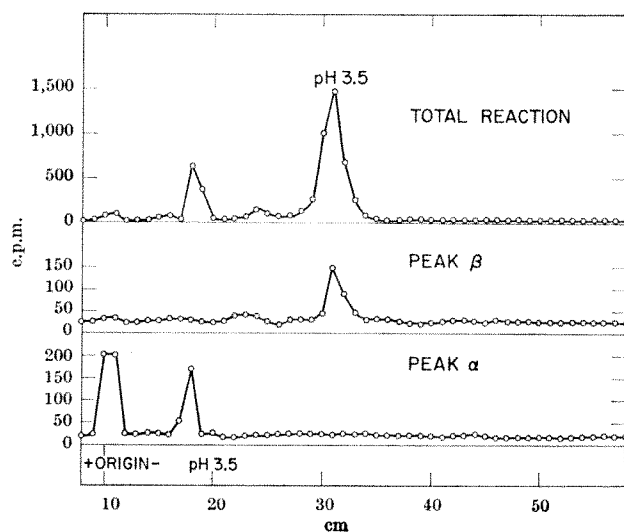


Fig. 5. Trypsin digest of formyl- ^{35}S -methionine-labelled α and β chains. Purified α and β chains labelled with formyl- ^{35}S -methionine, and also the total reaction product (Fig. 4) were digested with trypsin and analysed as in Fig. 2. The radioactivity at the origin in the peak α sample is undigested material.

Second, a reaction mixture containing formyl- ^{35}S -methionyl-tRNA^{MetF} was chromatographed on a column of 'Sephadex G-75'. Virtually all of the incorporated radioactivity chromatographed identically to the intact $\alpha_2\beta_2$ haemoglobin, measured by absorbance at 563 nm, present in the reticulocyte extract (Fig. 4a). This experiment suggests that formylmethionine is incorporated into intact, functional α and β chains and we confirmed this by the experiment in Fig. 4b. Globin chains were prepared from a similar *in vitro* reaction, and separated on a column of carboxymethyl (CM) cellulose. Two peaks of radioactivity were found; they eluted from the column slightly before the authentic α and β chain markers. CM-cellulose separates largely according to protein charge, so that this result is accounted for by incorporation of the formyl- ^{35}S -methionine radioactivity into α and β chains with a reduced positive charge, because of the blocked N-terminal amino group.

Finally, when digested with trypsin, the formyl- ^{35}S -methionine-labelled α and β chains each yielded a single radioactive peptide (Fig. 5). These have the expected ionophoretic mobility of the amino-terminal tryptic peptides of α and β chains containing an N-terminal formylmethionine. When these peptides were treated with dilute HCl to remove the formyl residue, they had an ionophoretic mobility consistent with the acquisition of a single positive charge (Fig. 6, top panel). Further characterization of these peptides is in progress, but we suggest that a formylmethionine residue can be incorporated at the amino terminus of both α and β chains, and that no other polypeptide beginning with formylmethionine is produced in our system.

Incorporation by Met-tRNA^{MetF}

The low level of methionine incorporation from ^{35}S -Met-tRNA^{MetF} (Fig. 1) could be explained by any of the following hypotheses: (1) Met-tRNA^{MetF}, like fMet-tRNA^{MetF}, is actually used for the initiation of haemoglobin synthesis, but the amino terminal methionine is rapidly removed from the nascent chain; (2) Met-tRNA^{MetF} is used for initiation of a protein synthesized in relatively small amounts, and (3) Met-tRNA^{MetF} inserts methionine into the internal positions of haemoglobin, but only poorly.

Evidence that both Met-tRNA^{MetF} and fMet-tRNA^{MetF} incorporate, respectively, methionine and formylmethionine into the same position of the polypeptide chain is derived from the experiment in Fig. 6. Protein synthe-

sized *in vitro* and labelled with ^{35}S -Met-tRNA^{MetF} was digested with trypsin and the product analysed by ionophoresis (middle panel). Two principal radioactive peptides were present and these had mobilities identical to those of the corresponding peptides from the product labelled with fMet-tRNA^{MetF} and from which the formyl groups had been removed by acid hydrolysis. Because no radioactive peptide having the mobility of the ^{35}S -Met-tRNA^{MetM} product—the internal methionine peptides of haemoglobin—was observed, we can also conclude that Met-tRNA^{MetF} probably transfers methionine only to the amino terminus of haemoglobin.

Table 2. SOURCE OF RADIOACTIVITY

	Formyl- ^{35}S -Met-tRNA ^{MetF} (106 $\mu\text{g}/\text{ml.}$)	^{35}S -Met-tRNA ^{MetF} (100 $\mu\text{g}/\text{ml.}$)	^{14}C -Lysine (1,162 c.p.m.)
No additions	1.00 (1,143 c.p.m.)	1.00 (82 c.p.m.)	1.00 (1,162 c.p.m.)
Plus fMet-tRNA ^{MetF} (76 $\mu\text{g}/\text{ml.}$)	Not done	0.25	0.81
Plus Met-tRNA ^{MetF} (76 $\mu\text{g}/\text{ml.}$)	0.19	0.23	0.79

Reactions are described in the legend to Fig. 1, except that those with ^{14}C -lysine contained no non-radioactive lysine, and 4 $\mu\text{Ci}/\text{ml.}$ ^{14}C -lysine (246 $\mu\text{Ci}/\text{mmole}$) and ^{35}S -methionyl-tRNA were added at 20 $\mu\text{g}/\text{ml.}$ Incubation was at 37° C for 10 min.

The experiment described in Table 2 also strongly indicates that Met-tRNA^{MetF} and fMet-tRNA^{MetF} compete for the same function—the initiation of haemoglobin synthesis. Thus (1) addition of Met-tRNA^{MetF} inhibits incorporation by formyl- ^{35}S -Met-tRNA^{MetF} and conversely non-radioactive fMet-tRNA^{MetF} inhibits incorporation by ^{35}S -Met-tRNA^{MetF}; and (2) the inhibition was specific, because the net level of haemoglobin synthesis—measured by incorporation of ^{14}C -lysine (Table 2) or ^{35}S -Met-tRNA^{MetM} (unpublished)—was not appreciably affected by either tRNA.

Furthermore, the low level of incorporation observed with ^{35}S -Met-tRNA^{MetF} as compared with formyl- ^{35}S -Met-tRNA^{MetF} suggests that ^{35}S -methionine incorporated from ^{35}S -Met-tRNA^{MetF} is removed from the nascent chain, whereas the formyl- ^{35}S -methionyl product is stable. A direct prediction of this is the conversion of a large amount of radioactivity from ^{35}S -Met-tRNA^{MetF} to free methionine.

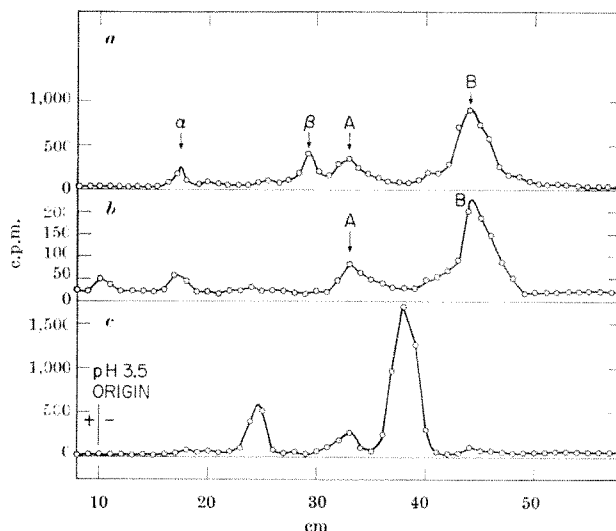


Fig. 6. Analysis of ^{35}S -Met-tRNA^{MetF} product. (a) Formyl- ^{35}S -methionyl-tRNA^{MetF} + HCl; (b) ^{35}S -methionyl-tRNA^{MetF}; (c) ^{35}S -methionyl-tRNA^{MetM}. Reactions containing ^{35}S -Met-tRNA^{MetF} (Fig. 1, 2.5 min at 25° C) or ^{35}S -Met-tRNA^{MetM} (10 min at 25° C) were digested and analysed as in Fig. 5, except the tRNA^{MetF} reaction was stopped by alkali (0.1 M KOH, 37° C, 10 min) and no ribonuclease was added. Protein labelled *in vitro* with formyl- ^{35}S -methionyl-tRNA^{MetF} and digested with trypsin was treated with HCl (0.5 M, 90° C, 20 min)²² to remove the formyl residues. α and β indicate the positions of the formylmethionine peptides from the α and β chains (Fig. 5); A and B are the deacylated derivatives, respectively, of these peptides.

To test this, a small amount of ^{35}S -Met-tRNA^{MetF} was added to a reticulocyte cell-free system (Fig. 7). Results show, first, that within 3 min more than 60 per cent of the radioactivity was converted to acid soluble form. Paper ionophoresis at pH 1.9 revealed that all of the ^{35}S radioactivity solubilized is indeed ^{35}S -methionine. Second, this acid solubilization requires protein synthesis on cytoplasmic 80S ribosomes, for it is inhibited by cycloheximide or aurintricarboxylate, but not by chloramphenicol. Third, this acid solubilization is specific, for in similar experiments with ^{35}S -Met-tRNA^{MetM} and formyl- ^{35}S -Met-tRNA^{MetF} there was less than 15 per cent formation of acid soluble radioactivity.

Table 3. INCORPORATION OF METHIONINE FROM ACETYL- AND FORMYL METHIONYL-tRNA

tRNA	Added	c.p.m./15 μl . aliquot Incorporation at 10 min	Incorporation at 30 min
Formyl- ^{35}S -methionyl tRNA ^{MetF}	12,000	1,368	2,423
Acetyl- ^{35}S -methionyl tRNA ^{MetF}	6,600	286	566
Acetyl- ^{35}S -methionyl tRNA ^{MetM}	8,200	15	27

Reactions are described in the legend to Fig. 1; incubation was at 25° C. Acetylation of Met-tRNA^{MetM} and Met-tRNA^{MetF} followed the procedure of ref. 34.

Table 3 shows that acetyl- ^{35}S -methionyl tRNA^{MetF} is also incorporated efficiently into protein, whereas acetyl- ^{35}S -methionyl-tRNA^{MetM} is not incorporated, so that the ability of formyl or acetyl Met-tRNA^{MetF} to initiate protein synthesis is not simply to be attributed to a blocked amino group, but rather to an intrinsic property of tRNA^{MetF}.

Steps in the Initiation Process

We have used yeast Met-tRNA^{MetM}, Met-tRNA^{MetF} and fMet-tRNA^{MetF} to synthesize haemoglobin in a rabbit reticulocyte cell-free system. Because all eukaryotic cells seem to contain the same two types of methionyl-tRNAs, the use of yeast tRNAs in these experiments should not affect the results. Furthermore, *E. coli* formylmethionyl-tRNA, the initiator tRNA of bacterial protein synthesis, is also incorporated by the reticulocyte cell-free system, albeit somewhat less efficiently than the yeast tRNA (unpublished results of D. H. and H. F. L.).

Our results support the following conclusions on the mechanism of initiation of haemoglobin synthesis. (1) Both haemoglobin chains are initiated *in vitro* with a methionine residue at their amino terminus. This methionine is subsequently removed, leaving the penultimate valine as the final amino terminus. (2) Met-tRNA^{MetF} inserts methionine into the amino-terminal position of haemoglobin chains, but cannot insert methionine into internal positions. (3) Met-tRNA^{MetM} inserts methionine only into the internal positions of haemoglobin. Even when the amino group of the methionine is blocked by an acetyl group, Met-tRNA^{MetM} cannot initiate haemoglobin synthesis. (4) Rabbit reticulocyte cell extracts contain an enzyme which can remove methionine from the amino terminus of haemoglobin. Blockage of the methionine amino group by a formyl or acetyl residue inhibits the action of this enzyme. Thus when N-formyl ^{35}S -methionyl-tRNA^{MetF} is used in the cell-free system, both haemoglobin chains contain a formyl methionine residue at their amino termini.

Bacterial protein synthesis always begins with the formylated amino-acid N-formylmethionine. That yeast and liver tRNA^{MetF} can be used to initiate bacterial protein synthesis, and that yeast and *E. coli* tRNA^{MetF} can initiate haemoglobin synthesis, strongly suggests that the unique properties of the initiator methionine tRNA were conserved during evolution. But in bacteria, the initiator methionyl-tRNA must contain a formyl residue in order to initiate protein synthesis. By contrast, there

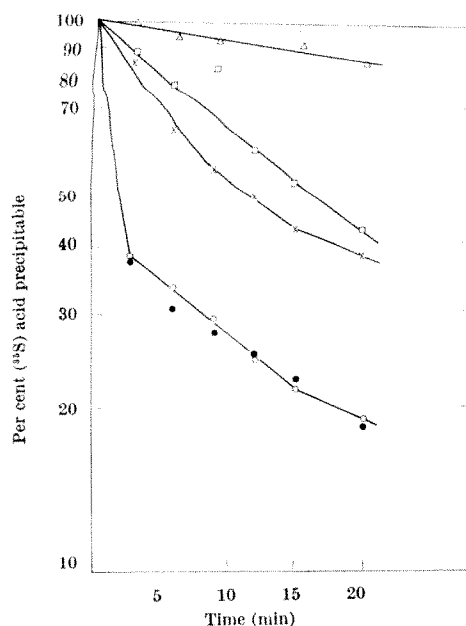


Fig. 7. Conversion of ^{35}S -Met-tRNA^{MetF} radioactivity to acid soluble form. Δ , Complete reaction; \bullet , plus chloramphenicol; \times , plus cycloheximide; \square , plus ATA. Reactions (Fig. 1) contained (where indicated) 200 $\mu\text{g}/\text{ml}$. cycloheximide, 60 $\mu\text{g}/\text{ml}$. chloramphenicol, 10^{-4} M aurintricarboxylate. A control reaction without reticulocyte extract was included. Incubation was at 37° C; 15 μl . aliquots were added to 0.5 ice-cold water, and immediately TCA to 5 per cent was added. Precipitates were collected and counted as in Fig. 1.

is no evidence for any formylated methionyl-tRNA in the cytoplasm of eukaryotic cells².

A further similarity between initiation of protein synthesis in bacteria and that of haemoglobin in rabbit reticulocytes is the fate of the N-terminal amino-acid in the final product. In *E. coli*, for instance, the N-terminal formyl methionine residue is removed in most cases through the successive use of two enzymes, a deformylase and a peptidase which removes the N-terminal methionine²⁴⁻²⁶. The observation that many bacterial proteins do contain N-terminal methionine indicates that the action of the bacterial aminopeptidase may be restricted by the nature of the amino-acid(s) adjacent to methionine. Comparison of haemoglobin sequences from several sources shows that methionine is present in the N-terminus of the β chains of beef and sheep haemoglobin²⁷. Experiments designed to show a direct incorporation of methionine from Met-tRNA^{MetF} into the β chains of sheep haemoglobin are now in progress.

Sherman *et al.* demonstrated by genetic techniques that yeast iso-1 cytochrome *c* has an AUG triplet (codon for methionine) proximal to the codon for the normal amino-terminal residue, threonine. No cytochrome *c* is produced when the AUG codon is mutated. Revertants of such mutations include second-site mutations to an AUG initiator codon at other positions in the gene; in particular they isolated several mutants which contained additional amino-acids, including an N-terminal methionine, at the beginning of the normal protein. Thus initiation of a protein produced on yeast cytoplasmic ribosomes seems also to require an AUG methionine codon^{17,18}, and it seems that initiation of protein synthesis by a special methionyl-tRNA may be a general phenomenon in the synthesis of all eukaryotic proteins.

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Factor Dependent Binding of Methionyl-tRNAs to Reticulocyte Ribosomes

by

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Reticulocyte initiation factors bind a formylatable methionine tRNA but not a non-formylatable tRNA. Elongation factors bind only the non-formylatable species. These findings suggest that haemoglobin chains may be initiated in a way similar to bacterial proteins.

PREVIOUS studies have indicated that three factors, M_1 , M_2 and M_3 , isolated from a 0.5 M KCl rabbit reticulocyte ribosomal wash fraction, are required in addition to the known polypeptide chain elongation factors, T_1 and T_2 , for cell-free haemoglobin synthesis at 3 mM Mg^{2+} (refs. 1-3). M_1 and M_2 can be assayed by their ability to lower the Mg^{2+} concentration optimum for poly U dependent polypeptide synthesis in the reticulocyte cell-free system, and this assay has provided a useful tool in the identification and isolation of these factors³. Because M_1 also stimulates the binding of both N-acetylphenylalanyl-tRNA and phenylalanyl-tRNA to reticulocyte ribosomes at low $[Mg^{2+}]$ ³, the role of the M factors in mammalian protein synthesis might be elucidated through the development of a triplet codon dependent binding assay based on differential responses of specific tRNAs to specific enzyme factors. Such an assay similar to the AUG and initiation factor directed binding of fMet-tRNA to washed *E. coli* ribosomes^{4,5,14} would also be useful in examining various tRNA species for special properties related to the mammalian initiation process.

Recently, Smith, Marcker and Brown^{6,7} described two species of methionine tRNAs from liver, "Met-tRNA_F" and "Met-tRNA_M", which are distinguished during cell-free ascites tumour protein synthesis in a manner similar to *Escherichia coli*: the latter inserts methionine into internal amino-acid positions of peptides and the former exclusively at the N-terminal position in response to either viral or "phased" synthetic oligonucleotide templates (oligonucleotides beginning with AUG at their 5' end). We describe here a mammalian triplet-dependent enzymatic binding assay using washed reticulocyte ribosomes, reticulocyte factors M_1 and M_2 , rabbit liver tRNA_F^{Met} and tRNA_M^{Met}, and the trinucleotide codon

AUG. In this system, Met-tRNA_F binds to ribosomes enzymatically at low $[Mg^{2+}]$ in response to M_1 plus M_2 , but poorly in response to T_1 . By contrast, Met-tRNA_M binds to washed reticulocyte ribosomes in response to T_1 , but not to M_1 plus M_2 . These data indicate that the factors M_1 plus M_2 can specify binding of the mammalian counterpart to a known bacterial initiator tRNA to the ribosome and can distinguish this tRNA from a non-initiator tRNA, which is acylated with the same amino-acid and responds to the same trinucleotide codon signal.

Triplet-Dependent Enzymatic Binding Assay

When we studied standard ribosomes washed with 0.5 M KCl for their ability non-enzymatically to bind

Table 1. REQUIREMENTS FOR BINDING OF Met-tRNA TO RETICULOCYTE RIBOSOMES AT 5 mM Mg^{2+}

Deletions or substitutions to reaction mixture	A pmoles ³ H-Met-tRNA bound	B pmoles ³ H-fMet-tRNA bound
Complete system	1.07	1.10
Minus M_1	0.30	0.16
Minus M_2	0.54	1.23
Minus M_1 and M_2	0.24	0.13
Plus ribosomal wash fraction	0.81	—
Plus 105,000g supernatant	0.37	—
Minus ribosomes	0.08	0.07
Minus AUG	0.40	0.25
Minus GTP	0.43	0.65
Minus M_2 , minus GTP	—	0.93

Experimental conditions are described in the legend to Fig. 1. Where indicated 78 μ g ribosomal wash protein or 0.5 mg 105,000g supernatant protein was added to the reaction mixture. In the complete system less than 0.08 pmoles of hot trichloroacetic acid precipitable material was retained on the nitrocellulose filter. (A) 10 pmoles of unfractionated rabbit reticulocyte ³H-Met-tRNA were used in each incubation. (B) 10 pmoles of unfractionated rabbit reticulocyte ³H-fMet-tRNA (60 per cent formylated) were used in each incubation.

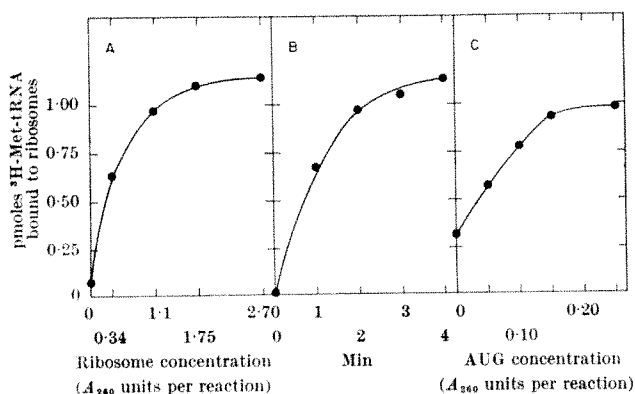


Fig. 1. Kinetic parameters for the triplet dependent enzymatic binding of ^3H -Met-tRNA to rabbit reticulocyte ribosomes at 5 mM Mg^{2+} . Reticulocyte ribosomes and the ribosomal wash factors M_1 and M_2 were prepared as previously described³ with the additional steps: ribosomes were washed a second time in standard sucrose (0.25 M sucrose, 1 mM dithiothreitol, 0.1 mM EDTA, potassium salt, pH 7.0) made 0.5 M in KCl; M_1 and M_2 were separately chromatographed on 'Sephadex G-150' after their original isolation by DEAE-cellulose chromatography (procedures as described in ref. 3). The sequenced trinucleotide ApUpG was purchased from Miles Laboratories; high voltage electrophoresis revealed one major and four minor bands. The major band represented authentic AUG and contained approximately 50 per cent of the total A_{260} units in the preparation. Enzymatic binding of ^3H -Met-tRNA to washed reticulocyte ribosomes was performed by a modification of the nitrocellulose filter assay originally reported by Nirenberg and Leder¹⁴. Incubations, in a total volume of 50 μl ., were performed at 23°C and contained 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 5 mM MgCl_2 , 0.2 mM GTP (neutralized to pH 7.0 with KOH), 1 mM dithiothreitol, 10 pmoles ^3H -Met-tRNA (unfractionated rabbit reticulocyte tRNA¹³ acylated 3.4 per cent with L- ^3H -methionine, specific activity 2,500 mCi/mole), 15 μg M_1 protein, 6.3 μg M_2 protein, and ribosomes and AUG concentration as indicated. The reactions were stopped by addition of 3 ml. of wash buffer (20 mM Tris-HCl (pH 7.5)—100 mM KCl—5 mM MgCl_2 , 4°C). The reaction mixture was then immediately filtered on a nitrocellulose filter, washed three times with 3 ml. wash buffer at 4°C, and counted in a liquid scintillation spectrometer as previously reported³. A blank of 0.03 pmoles, representing the activity observed in the absence of both ribosomes and factors, was subtracted from each point. A, Ribosome concentration curve determined at a 2 min time point with 0.15 A_{260} units of AUG. B, Time course of reaction determined with 1.1 A_{260} units of ribosomes and 0.15 A_{260} units of AUG. C, AUG concentration curve determined at a 2 min time point with 1.1 A_{260} units of ribosomes.

unfractionated reticulocyte ^3H -Met-tRNA at high $[\text{Mg}^{2+}]$, little activity was obtained with the triplet AUG. When twice washed reticulocyte ribosomes were used, however—see legend to Fig. 1 for experimental procedures—activity was markedly increased. We then found that similar triplet dependent binding could be obtained enzymatically at low $[\text{Mg}^{2+}]$ by introducing the crude ribosomal wash fraction (Table 1A). Crude supernatant or partially purified reticulocyte T_1 worked poorly. M factors, which had been completely separated from T_1 and T_2 by a combination of DEAE-cellulose and 'Sephadex G-150' chromatography, were then tested for their ability to replace crude ribosomal wash in AUG dependent enzymatic binding of unfractionated ^3H -Met-tRNA. M_1 could partially replace the crude wash fraction, but activity was markedly enhanced by the further addition of M_2 . This AUG mediated binding of Met-tRNA to reticulocyte ribosomes at 5 mM Mg^{2+} was almost completely dependent on GTP (Table 1A).

Table 1B shows the influence of an N-formyl group on the binding reaction. When formylated, unfractionated methionine tRNA (fMet-tRNA, approximately 60 per cent formylated) was used as substrate, there was a minimal increase in binding in the presence of M_1 plus M_2 , and AUG dependence remained unchanged. But, with this substrate (fMet-tRNA), there was a complete loss of the M_2 requirement, a partial loss of GTP dependence, and a rise in the $[\text{Mg}^{2+}]$ optimum for enzymatic binding (data not shown).

The kinetic curves for the ribosome concentration, time, and AUG concentration which characterize the triplet dependent enzymatic binding assay, are given in Fig. 1A, B and C. In subsequent studies, 1.1 A_{260} units of ribosomes, a 2 min reaction time, and 0.15 A_{260} units

of AUG were used. Using unfractionated rabbit liver ^3H -Met-tRNA as substrate, we performed enzyme concentration studies at 5 mM Mg^{2+} for both M_1 and M_2 (Fig. 2A, B). Significant triplet mediated activity was obtained with M_1 alone, but not with M_2 alone. Maximum activity required both M_1 and M_2 . Saturating enzyme levels, 15 μg M_1 protein and 6.3 μg M_2 protein per 50 μl . reaction mixture, were used in all subsequent studies.

Factor Dependent Binding of Met-tRNA^f and Met-tRNA^{Met}

To examine factor specificity in the binding of the two types of methionyl-tRNA, that is, formylatable (tRNA^{fMet}) and non-formylatable (tRNA^{Met}), the tRNA^{Met} species from rabbit liver tRNA were separated by 'Freon' reversed phase (RPC-2) chromatography^{8,9}. Fractions containing tRNA^{Met} were identified by acylation with crude reticulocyte synthetase, and three principal peaks were obtained (Fig. 3). The Met-tRNA from fractions 15 (peak I), 17 (peak II) and 21 (peak III) were then tested for their ability to be formylated with crude *E. coli* transformylase¹⁰. After a 30 min incubation period, 100 per cent of the Met-tRNA in peak I and 88 per

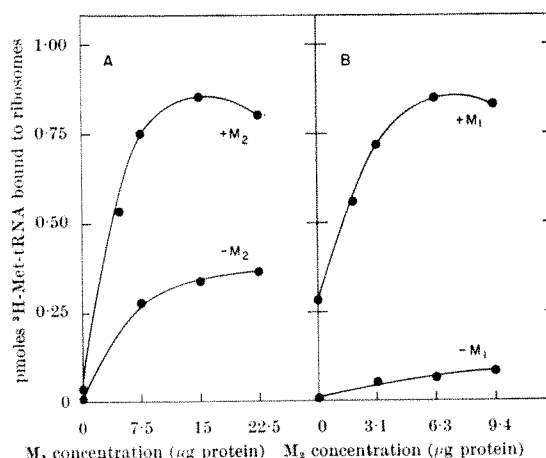


Fig. 2. M_1 and M_2 enzyme saturation curves for AUG dependent binding of ^3H -Met-tRNA to reticulocyte ribosomes at 5 mM Mg^{2+} . Experimental details are described in the legend to Fig. 1 and in the text. A blank of approximately 0.29 pmoles, representing the sum of the activities obtained with ribosomes alone and with M_1 and/or M_2 as a function, was subtracted from each value. A, ^3H -Met-tRNA binding as a function of M_1 concentration (μg protein per 50 μl . reaction mixture). Upper curve: in the presence of 6.3 μg M_2 protein; lower curve: in the absence of M_2 . B, ^3H -Met-tRNA binding as a function of M_2 concentration. Upper curve: in the presence of 15 μg M_1 protein; lower curve: in the absence of M_1 .

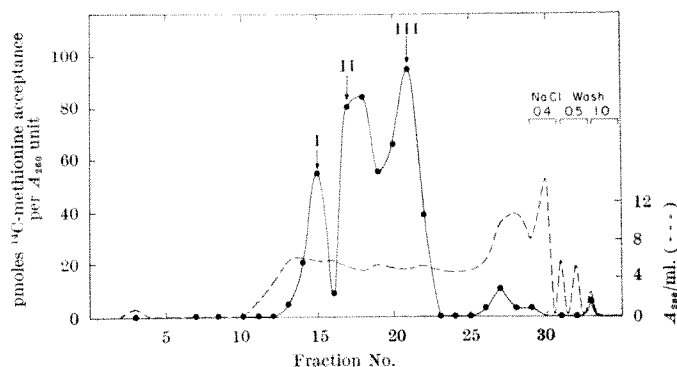


Fig. 3. Fractionation of rabbit liver tRNA by 'Freon' reversed phase chromatography (RPC-2). Details of this preparative column have been published⁹. Column fractions, which had been stored lyophilized at -20°C, were tested for methionine acceptance as described in the text. The arrows indicate the three fractions tested for their ability to be formylated (see text). The material from fractions 17 and 21 was used for the studies shown in Figs. 4 and 5, respectively.

cent in peak II were formylated, whereas only 7.9 per cent of the Met-tRNA in peak III was formylated. The ability of each Met-tRNA species to bind enzymatically to reticulocyte ribosomes in response to AUG was then tested. Both peaks I and II (Met-tRNA_I) were enzymatically bound to ribosomes with M₁ plus M₂, whereas peak III was inactive. By contrast, when T₁ was used as the binding enzyme, only peak III (Met-tRNA^{Met}) was actively bound to ribosomes. Figs. 4 and 5 show curves for the dependence on [Mg²⁺] of the binding with Met-tRNA_I (peak II) and Met-tRNA^{Met} (peak III), respectively. With Met-tRNA_I as substrate (Fig. 4A), M₁ plus M₂ are most active at low [Mg²⁺]; T₁ is poorly active at low [Mg²⁺], but becomes progressively more active as [Mg²⁺] is raised toward 10 mM. These observations become more apparent when the contribution by non-enzymatic binding (that obtained in the absence of added enzyme factors not distinguishing between possible ribosomal binding sites) is subtracted from total bound radioactivity (Fig. 4B). With this substrate (Met-tRNA_I), the binding reaction with T₁, as well as that with M₁ plus M₂, is dependent on both AUG and GTP. When Met-tRNA^{Met} is used (Fig. 5A, B), T₁ is highly active, M₁ poorly active (data not shown) and M₁ plus M₂ totally inactive. In the conditions used, the enzymatic binding of Met-tRNA^{Met} to ribosomes with T₁ is GTP dependent, but not AUG dependent.

Specificities

The studies by Smith, Mareker and Brown^{6,7} indicated that Met-tRNA_I may be a specific initiator tRNA in the ascites tumour cell-free system in response to both artificial and viral messages. But this does not mean that Met-tRNA_I is the only mammalian initiator. In other reports, several N-blocked aminoacyl-tRNAs have been suggested as possible initiators in various mammalian systems: N-blocked valine-tRNA for haemoglobin¹¹, PCA-tRNA for immunoglobulins¹², and N-acetylserine-tRNA for histones¹³. In this investigation, using our mammalian triplet dependent enzymatic tRNA binding assay, we have found that Met-tRNA_I specifically responds to M₁ plus M₂ at low [Mg²⁺] and the reaction is both AUG and GTP dependent. There is no apparent requirement for a blocking group, although this does not mean that a blocking group is not formed transiently during the incubation period. With Met-tRNA^{Met} as

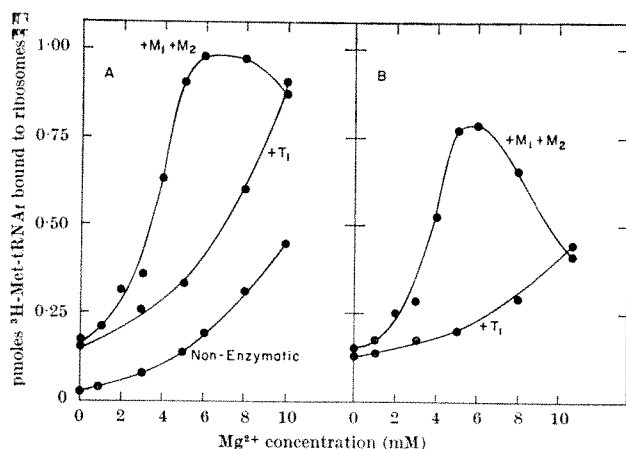


Fig. 4. Mg²⁺ dependence for AUG-dependent binding of ³H-Met-tRNA_I to reticulocyte ribosomes. The source of the tRNA^{Met} was fraction 17 from Fig. 3. Each 50 μ l reaction mixture contained 8 pmoles ³H-Met-tRNA_I (L-³H-methionine, specific activity 2,500 mCi/nmole), 24 μ g T₁ protein where indicated, and other components as listed in the legend to Fig. 1. For each point, the wash buffer contained the same [Mg²⁺] as the reaction mixture. A blank of 0.03 pmoles, representing the activity observed in the absence of both ribosomes and factors, was subtracted from each point. A, ³H-Met-tRNA_I binding to reticulocyte ribosomes in the presence of M₁ plus M₂, T₁, or in the absence of added factors (non-enzymatic binding). B, Same data as in A but with the contribution by non-enzymatic binding subtracted from each point.

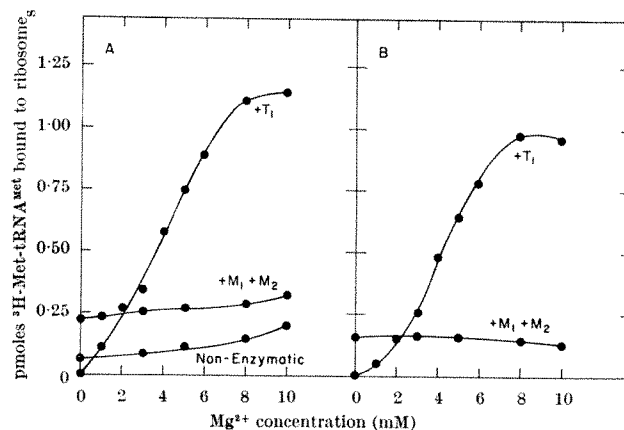


Fig. 5. Mg²⁺ dependence for the binding of ³H-Met-tRNA^{Met} to reticulocyte ribosomes. The source of the Met-tRNA^{Met} was fraction 21 in Fig. 3. Experimental conditions were the same as for Fig. 4. A, ³H-Met-tRNA^{Met} binding to reticulocyte ribosomes in the presence of T₁, M₁ plus M₂, or in the absence of added factors (non-enzymatic binding). B, Same data as in A but with the contribution by non-enzymatic binding subtracted from each point.

substrate, there is a reversal of enzyme specificity, so that activity is obtained with T₁ rather than M₁ plus M₂. This reaction is dependent on GTP but not, apparently, on AUG.

The exact relationship of this binding assay to the role of M₁ and M₂ in reticulocyte protein synthesis is still not clear. In appropriate conditions, M₁ alone seems to recognize various blocked as well as unblocked aminoacyl-tRNAs (ref. 3 and unpublished observations). M₂ alone, on the other hand, is inactive with all tRNAs tested. The loss of M₂ as well as GTP dependence for the binding of fMet-tRNA by contrast with that of Met-tRNA (as shown in Table I) is also not clear.

Our findings indicate that in appropriate experimental conditions, reticulocyte ribosomes can be utilized in a triplet dependent, enzymatic tRNA binding assay and that the tRNA species, Met-tRNA_I, and the enzyme factors, M₁ and M₂, have roles in the reticulocyte system compatible with the known mechanism for the initiation of protein synthesis in bacterial cells. These results cannot, however, be taken as proof that Met-tRNA_I is a natural initiator in the reticulocyte cell-free system, because the tRNA species and/or the enzyme factors could conceivably represent only vestigial activities in the mammalian system. We hope to determine whether the initiation of haemoglobin synthesis in a tRNA-dependent cell-free haemoglobin synthesizing system depends on the addition of tRNA^{Met}, to examine other tRNAs for initiator properties, to look for initiation specific peptide bond formation, and to use natural haemoglobin messenger as the initiator signal in the binding assay.

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Reactions of Haemoglobin Dimers after Ligand Dissociation

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The absorption spectra of haemoglobin solutions containing varying proportions of tetramer and dimer were followed immediately after the removal of oxygen. Two parallel reactions involving conformational transitions contribute to the observed spectral change: a first order reaction of the dimer which need not be cooperative and a very much faster reaction of tetramer which can be controlled by the second order association of dimer to tetramer.

For more than sixty years, theoretical and experimental investigations of haemoglobin have been concerned with the study of the nature of the molecular interactions which result in the cooperative binding of oxygen¹. It is this property of haemoglobin which, by narrowing the range of the oxygen pressure controlling uptake and release, is responsible for its efficiency in oxygen transport.

In the erythrocyte, haemoglobin occurs as a tetramer, consisting of two α and two β chains, and may exist as either a constrained or an unconstrained form in the absence or presence of ligand respectively. The constrained tetramer is characterized by a lower affinity for and a slower rate of combination with ligand than the unconstrained tetramer². Further, the X-ray studies of Perutz and his collaborators have shown in remarkable detail how the quaternary structures of the two forms differ^{3,4}. It is the change in conformation from one structure to the other on ligand binding which forms the basis of the cooperative phenomena. In solution studies, therefore, our principal interest must be the elucidation of the sequence of events within the tetramer which bring about the conformational change.

While this problem remains, another important question has been debated in the past twenty years⁵. In the concentration range (10^{-2} – 10^{-4} M haem) normally used to study haemoglobin reactions, dissociation of the tetramer to dimer occurs. At present no agreement has been reached concerning whether or not there are distinct properties of the dimer and tetramer in their reactions with ligand^{2,6–14}. Here this question is approached by correlating kinetic features with the fraction of dimer determined from measurements of the tetramer–dimer dissociation equilibrium. Although there is general agreement on the value of equilibrium constants for the liganded molecule^{10,15–20}, there are conflicting data concerning the non-liganded molecule^{2,10,17,19}. For the necessary correlation it was decided therefore to investigate the slow spectral change^{21,22} occurring immediately after removal of ligand in two sets of conditions, one in which tetramers and dimers coexist and one in which there is essentially all dimer.

To describe these reactions of haemoglobin in dilute solution we propose a simple kinetic model in which two reactions contribute to the slow spectral changes observed in dilute salt and neutral pH. The first reaction is a slow first order process ($t_1 \approx 3.5$ s) representing a conformational change within the dimer. The second reaction is the extremely fast ($t_2 < 100$ μ s) conformational change of the tetramer. In the experimental conditions, however, this reaction is kinetically controlled by the slow second order association of dimer to tetramer. The reason that the slow first order process has not been previously identified in haemoglobin reactions, even though the consequences of conformational transition are well known, is that it is masked by the second order dimer–dimer association reaction which occurs in parallel. It is shown that the

proposed model is consistent with a wide range of experimental haemoglobin data presented in the literature.

"Base-line Drift"

In all experiments reported here, the removal of the ligand oxygen has been effected by mixing oxyhaemoglobin in the stopped-flow apparatus with a solution of sodium dithionite. This solution, in which sodium dithionite was at a concentration of 2 mg/ml. before mixing, was prepared by dissolving the solid in deoxygenated buffer. The pH of the buffer was adjusted with NaOH before the addition of dithionite to compensate for the increase in acidity caused by solution of the dithionite.

Fig. 1 shows a stopped-flow trace recorded at 429 nm on the removal of oxygen from oxyhaemoglobin in 1 M NaI (pH 7.0) buffer at a concentration of 6.2μ M haem before mixing. The removal of oxygen, represented by the curve AB, is complete after about 100 ms. But the base-line recorded after about 60 s, represented by CD, is not coincident with that observed immediately after the initial removal of oxygen. Fig. 2a shows the recording of this difference observed in 1 M NaI at high sensitivity. Such base-line drifts have been noted previously (Gibson and Roughton, unpublished data quoted by Gibson²¹), and drifts of a similar nature, though over a longer time scale, have been attributed to side effects caused by the use of dithionite.

Antonini *et al.*, however, in their key paper²² on the kinetics of haemoglobin reactions, have concluded that such drifts occurring within seconds of the removal of oxygen by dithionite, are in fact real kinetic features of the haemoglobin system. Evidence for this conclusion is afforded in a general sense by the reproducibility of

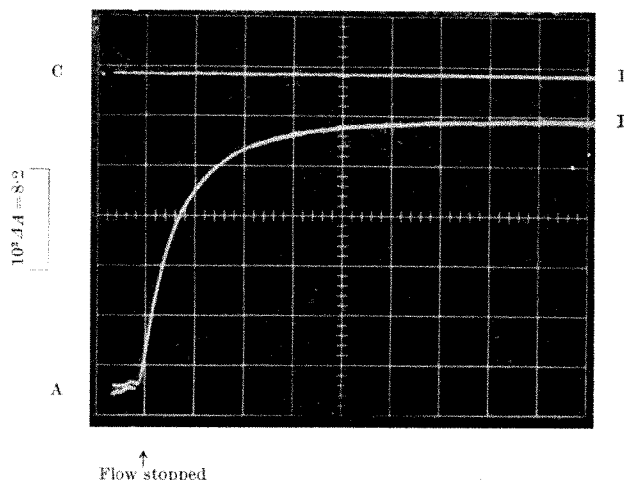


Fig. 1. Stopped-flow record of the rapid deoxygenation of oxyhaemoglobin in 1 M NaI (pH 7.0). Concentrations before mixing, 6.2μ M haem and 2 mg/ml. dithionite; 1 cm grid, scan time 50 ms/cm; 429 nm.

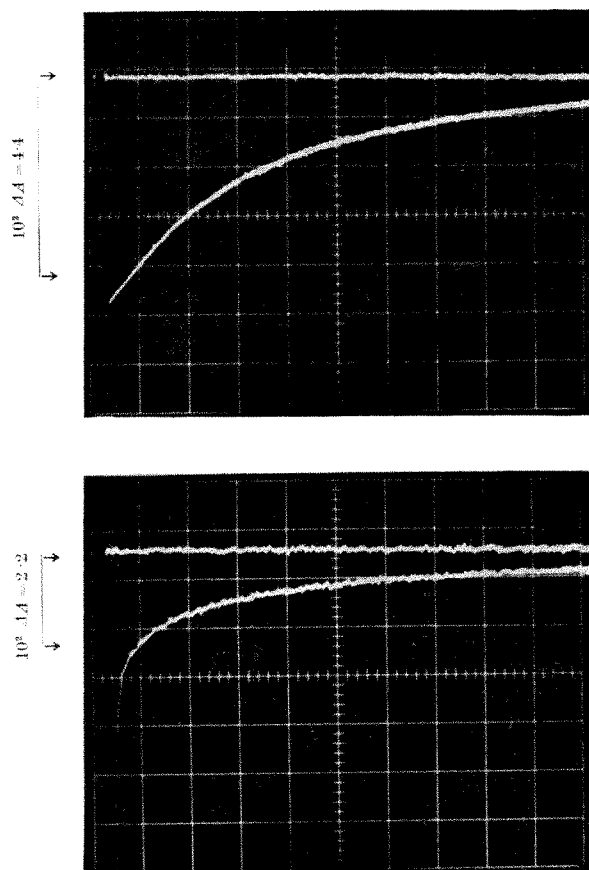


Fig. 2. *a*, Amplification of the "drift" phase BD (Fig. 1) in 1 M NaI (pH 7.0). Scan time 2 s/cm; other conditions as for Fig. 1. *b*, "Drift" phase observed at the same concentration as for *a* in 0.09 M NaCl (pH 7.0). Concentrations before mixing, 6.2 μ M haem and 2 mg/ml dithionite; scan time 0.5 s/cm; 429 nm.

both the amplitude and the velocity of the drift. More specifically, it is shown in control experiments repeated here with single chains, α^{SH} , that no such drifts occur at neutral pH either in 0.09 M NaCl or in 1 M NaI. Thus they cannot be caused by side effects of dithionite. Further, there is a characteristic difference spectrum in the Soret region between unliganded haemoglobin before and after the drift (Fig. 3). The maximum observed at 429 nm was used to follow the reactions here. The drift cannot be caused by slow oxygen dissociation from sites of abnormally high affinity since the difference spectrum is distinct in form from that between oxy and unliganded haemoglobins. The conclusion reached by Antonini *et al.*²² that this base-line drift is in fact a real kinetic feature of the haemoglobin system is amply confirmed by the experiments discussed here.

A Dimer Reaction expressed by the "Drift"

To identify the "drift" reaction with a particular species, it is necessary to have a detailed knowledge of the tetramer-dimer dissociation equilibrium of oxyhaemoglobin in the two sets of experimental conditions used in this investigation, namely 1 M NaI and 0.09 M NaCl at pH 7.0 (0.01 M Tris, 10^{-3} M EDTA). In similar conditions, the formation of monomer²⁴ in dilute solutions (10^{-6} to 10^{-7} M haem) has been shown to be irreversible and to be inhibited by 10^{-3} M EDTA (unpublished results of G. L. K.). In the presence of EDTA, the tetramer-dimer dissociation equilibrium constant, $K_{4,2}$, was then determined by sedimentation equilibrium to be 2.9 μ M haem in 0.09 M NaCl (pH 7.0) in good agreement with the value of 2.5 μ M haem from gel-filtration studies²⁰. In 1 M NaI, $K_{4,2}$ was tentatively estimated to be about 6.2 mM haem.

Thus within the present range of kinetic observation from 2.1 to 16 μ M haem, oxyhaemoglobin is completely in the form of dimer in 1 M NaI, whereas in 0.09 M NaCl the fraction of dimer varies between about 0.65 and 0.35.

Fig. 4 shows that the amplitude of the "drift" phase is directly proportional to oxyhaemoglobin concentration in 1 M NaI. To determine whether or not the "drift" phase may be correlated with either the dimer or the tetramer, the concentration dependence of its amplitude was then studied in 0.09 M NaCl (pH 7.0). In these conditions at a concentration of 6.2 μ M haem, the amplitude of the "drift" phase was found within experimental error to be half (Fig. 2*b*) of that found in 1 M NaI (Fig. 2*a*). At this concentration oxyhaemoglobin is 50 per cent dissociated into dimer in 0.09 M NaCl and completely dissociated in 1 M NaI, so that the "drift" phase must be correlated with the dimer.

The fraction of dimer was therefore calculated at each concentration examined by comparing the amplitude in 0.09 M NaCl with that in 1 M NaI at the same total concentration. The fraction of dimer versus the total concentration before mixing is shown in Fig. 5. The concentration before mixing was used in this correlation for the dissociation rate of oxyhaemoglobin from tetramer to dimer in dilute salt is relatively slow ($t_{1/2}$ of the order of 1 s) compared with the rate of oxygen dissociation. Thus the removal of oxygen was effected here before any detectable increase could be observed in the fraction of dimer over that present before mixing due to dissociation upon dilution in the stopped-flow apparatus. The solid curve in Fig. 4 is drawn for a tetramer to dimer dissociation constant of 3.1 μ M haem. This compares well with the values of 2.5 and 2.9 μ M haem determined by gel-filtration and sedimentation equilibrium, respectively, in the same conditions. The correlation of the slow phase with the dimer clearly implies that the equivalent spectral change due to the tetramer occurs relatively quickly, and indeed in the conditions expressed in Fig. 5 must be complete with the completion of oxygen removal.

At low oxyhaemoglobin concentrations, plots of $\ln \Delta A$ versus t are close to linearity. In the initial stages of the "drift" phase, there is present in the plot some slight curvature, which becomes pronounced only at higher concentrations. In the latter instance, plots of $1/\Delta A$ versus t are close to linearity in the initial stages and deviate only in the later stages. The combination of these features is evident in the biphasic nature of the "drift" phase seen at 6.2 μ M haem in 0.09 M NaCl (Fig. 2*b*). These observations suggest strongly that the principal reactions of the slow phase may well be first and second order reactions of the dimer and its products producing similar spectral changes.

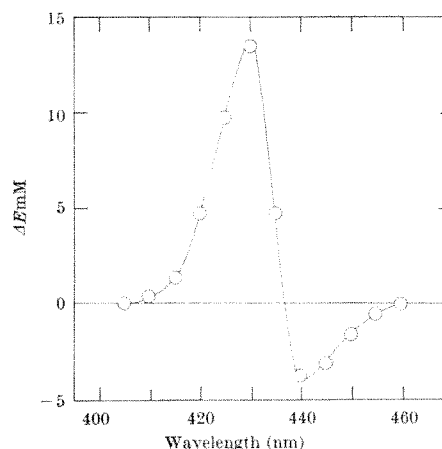
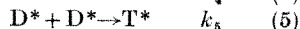


Fig. 3. Spectrum of the change in extinction, ΔE_{mM} (haem), represented by the "drift" phase in 1 M NaI (pH 7.0). Observations were made at a concentration of 6.2 μ M haem after mixing.

Kinetic Model for the "Drift" Phase

The dimer produced by the dissociation of oxyhaemoglobin tetramer in dilute salt at neutral pH is the $\alpha_1\beta_1$ dimer^{3,25-27}. The principal feature of the following reaction sequence is that it assumes that the $\alpha_1\beta_1$ dimer may undergo a conformational change. In this scheme, species having the characteristic conformation of the final constrained, unliganded form at equilibrium are denoted by an asterisk, whereas the species in the initial unconstrained, liganded form have no asterisk.



Immediately on removal of ligand, the unliganded dimer so produced has the unconstrained, liganded conformation, D. This may then undergo a conformational transition represented by reaction 1 to the constrained conformation, D*. The constrained dimer may subsequently associate with another one to form the constrained tetramer, T*, by reaction 5. The reverse reaction 6 must also exist, however slow this may be compared with reaction 5. Alternatively, rather than undergo the conformational transition 1, the unconstrained, unliganded dimer, D, may associate with a like dimer to produce an unconstrained tetramer, T, expressed in reaction 2. This tetramer may then either dissociate according to reaction 3, so defining the dissociation-association equilibrium characteristic of the fully liganded molecule, or it may change by reaction 4 to the constrained tetrameric conformation, T*.

We assume that reactions 5 and 6 involve no spectral changes and are consequently not seen in the "drift" phase. This assumption means that no knowledge of the dissociation-association equilibrium between the constrained tetramer and dimer is required for this analysis. In these circumstances, the problem is primarily resolved by a comparison of the rate of dissociation of the unconstrained tetramer, reaction 3, with the rate at which the tetramer changes conformation, reaction 4. At neutral pH in dilute salt, the rate of dissociation of liganded, unconstrained tetramer is relatively slow. The value of t_4 is approximately 1.4 s in 0.1 M phosphate (pH 7.0)^{22,28}. By comparison, the rate of the tetramer conformational change is a fast process: $t_4 < 1$ ms. Thus the "drift" phase correlates simply with the fraction of dimer, implying that the equivalent tetrameric change is completed with the fast removal of ligand. Alternatively, the fraction of "rapidly reacting haemoglobin" correlates directly with dimer in dilute salt at neutral pH¹³, showing that the tetramer changes conformation more quickly than ligand

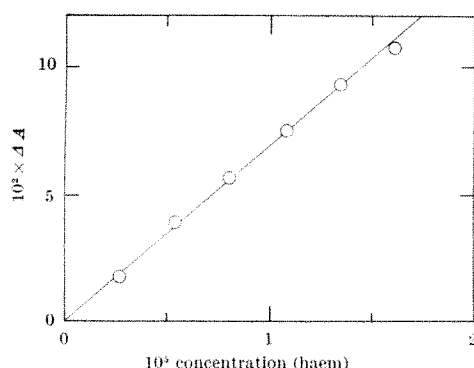


Fig. 4. The amplitude (ΔA) of the "drift" phase as a function of total haemoglobin concentration (haem) before mixing in 1 M NaI (pH 7.0).

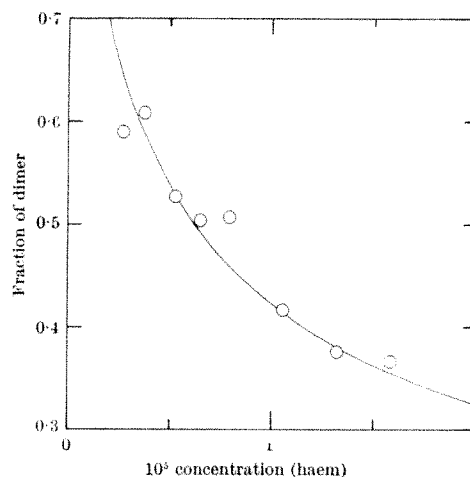


Fig. 5. The fraction of dimer as a function of total concentration before mixing in 0.09 M NaCl (pH 7.0). The fraction of dimer (\circ) was determined by comparing the amplitude of the "drift" phase in 0.09 M NaCl with that in 1 M NaI at the same total concentration. The solid curve is calculated for a dissociation constant of $3.1 \mu\text{M}$ haem.

can combine with the tetramer even in its rapidly reacting form. Further, it has been shown by Antonini and Brunori²⁹, using the change in reactivity of the β -93 sulphhydryl group as an index of conformation change, that the transition from constrained to unconstrained conformation must occur within a time rather less than the dead-time of a stopped-flow apparatus, that is, less than 1 ms.

In dilute salt at neutral pH therefore, if reaction 2 occurs, reaction 4 will always occur in preference to reaction 3 and reaction 2 will be the rate-limiting step. The reaction scheme then reduces simply to equations 1 and 2



For this simple case of parallel first and second order reactions producing the same spectral change, it is readily shown that

$$t_4 = \frac{1}{k_1} \ln \left[\frac{k_2 + 0.5 k_2 D_0}{k_1 + k_2 D_0} \right] + \frac{\ln 2}{k_1} \quad (7)$$

where D_0 is the concentration of the unconstrained dimer at time $t=0$. Fig. 6 shows the concentration dependence of t_4 observed in 0.09 M NaCl (pH 7.0) analysed in terms of equation 7. The concentration of dimer (expressed in μM haem) was calculated from the fraction of dimer at the haemoglobin concentration before mixing. The concentration so calculated was then halved before the application of equation 7 to correct for dilution in the cell. The solid curve was computed with the values of k_1 and k_2 of 0.20 s^{-1} and $4.3_5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ respectively. Because k_2 is the association rate constant of unconstrained dimers, it is now possible to calculate k_3 , the dissociation rate constant, from a knowledge of the dissociation equilibrium constant, $3.1 \mu\text{M}$ haem, in 0.09 M NaCl (pH 7.0). The value of k_3 is found to be 1.3_6 s^{-1} corresponding to t_3 for the dissociation reaction of approximately 0.5₁ s.

The Fast Dimer is Slow

We have presented kinetic observations of spectral changes in haemoglobin solutions immediately after the removal of ligand. They provide evidence for a first order reaction characterizing a slow conformational change of the dimer which reacts rapidly with ligand in flash photolysis experiments, and for a very much faster

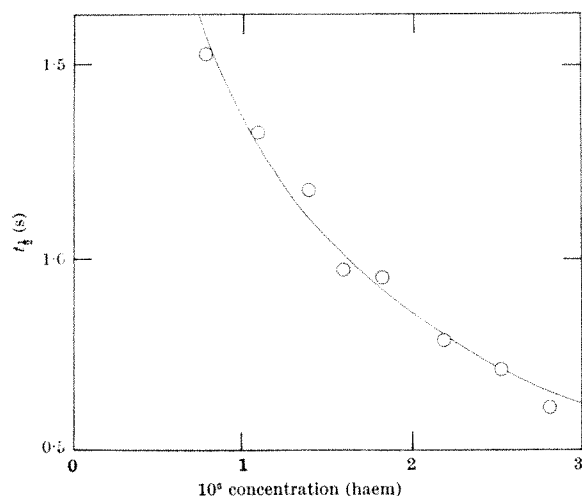


Fig. 6. The dependence of t_d for the "drift" phase in 0.09 M NaCl (pH 7.0) on the concentration of dimer (see text). The solid curve is calculated by the application of equation 7 using values of k_1 and k_2 of 0.20 s^{-1} and $4.3 \times 10^5 \text{ M (haem)}^{-1} \text{ s}^{-1}$.

change within the tetramer. We now wish to show how the present results can be used to interpret some of the interesting experiments reported in the literature on the behaviour of solutions containing varying proportions of tetramer and dimer.

After the flash photolysis of carboxyhaemoglobin the recombination curve of haemoglobin with CO is biphasic³⁰, the fast phase representing a reaction some forty times faster than that of the slow phase. The first observation is that in dilute salt at neutral pH the percentage of "rapidly reacting haemoglobin" correlates directly with the percentage of dimer¹³ (see Fig. 5). This rapid rate, however, is very similar to that of the isolated α and β chains. These are well known not to undergo a conformational transition on ligand binding and they thus exhibit hyperbolic saturation curves characteristic of a non-cooperative binding process. Using this criterion as an index of the absence of cooperativity, it has been concluded that the dimer is devoid of cooperativity¹³. But clearly such a correlation is also to be expected on the basis of the present finding that the conformational change within the dimer and the conversion of dimer to slowly reacting tetramer are relatively slow processes. The correlation follows directly from the fact that the rate of the tetramer conformation change, $t_4 < 100 \mu\text{s}$, is much faster than the rate at which ligand binds with "rapidly reacting haemoglobin"— t_2 generally of the order of 2 to 10 ms—which in turn is much faster than the rate at which the dimer either changes conformation or associates to tetramer: t_1 of the order of seconds. Thus the initial tetramer changes to its slowly reacting form almost instantaneously on flash photolysis before it can recombine with ligand; the ligand combines preferentially with the dimer in the unconstrained, rapidly reacting conformation, and then subsequently with slowly reacting tetramer.

This sequence of events also explains why the percentage of "rapidly reacting haemoglobin" decreases²² with increasing time of illumination causing dissociation of CO from carboxyhaemoglobin. The increased time over a period of seconds simply allows more time for the dimer to change to a constrained form which associates strongly to the slowly reacting, constrained tetramer or for the dimers to associate before the fast tetramer conformation change from the unconstrained form. A plot of the percentage of "rapidly reacting haemoglobin" against length of illumination is thus equivalent to observing the "drift" phase directly. This situation is represented in Figs. 3 and 6 of the article by Antonini *et al.*²².

The second observation is that the percentage of "rapidly reacting haemoglobin" in high salt solutions at neutral pH or in acetate solutions is always less (as much as 20 per cent less) than the percentage of dimer by contrast with the situation in dilute salt at neutral pH. This observation stems at least in part from the fact that the second order association step of unliganded, unconstrained dimers, reaction 2, becomes competitive with rate at which ligand binds to "rapidly reacting haemoglobin". Experiments designed to correlate a reaction property with the dimer species are necessarily performed at concentrations around the equilibrium dissociation constant of tetramer to dimer. In 0.09 M NaCl (pH 7.0); 2 M NaCl (pH 7.0); and 0.25 M acetate (pH 5.4), the half-dissociation points are 6.2 μM , 82.3 μM and 0.56 mM in haem, respectively. Simple steric considerations^{3,31} suggest that the association rate constant of unconstrained dimers ($4.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ in 0.09 M NaCl, pH 7.0) is similar for each condition, so that if the fast reacting dimer disappears entirely through the self-association step, the t_1 values for each half-dissociation point are 0.74 s, 56 ms and 8.2 ms, respectively. Within the concentration range investigated here, the value of t_1 observed in conventional flash photolysis experiments for the combination of CO with "rapidly reacting haemoglobin" varies from approximately 10 ms in dilute salt to about 2 ms in concentrated salt solutions.

Thus within the concentration range of each experiment, the t_1 in dilute salt for the second order self-association step is very much slower than that for the rapid combination of ligand whilst the rates of the two processes in high salt and acetate become comparable. Thus in these cases, particularly so for acetate, the second order process actually converts some rapidly reacting dimer to slowly reacting tetramer, by way of reactions 2 and 4, before the ligand can react with dimer to result in the observation that the percentage of "rapidly reacting haemoglobin" is always less than that of the dimer.

Two Kinds of Dimer ?

The long held persuasion that the dimer possesses the full cooperative characteristics of haemoglobin has rested primarily on the observation that the ligand binding characteristics of haemoglobin are not significantly affected by changes in conditions known to alter the tetramer-dimer equilibrium of oxyhaemoglobin^{2,5-12,14}. In particular, the value of n in the Hill plot remains close to 2.7 even in conditions where the tetramer is believed to be substantially dissociated to dimer in both the unconstrained and constrained forms^{10,17,18}.

The postulated "cooperative dimer" has been reconciled¹² with evidence from flash photolysis of a "non-cooperative" dimer¹³ by the suggestion that the unconstrained and constrained tetramers dissociate across different planes to form alternatively $\alpha_1\beta_1$ and $\alpha_1\beta_2$ dimers (Perutz notation), characterized by fast and slow reaction with ligand respectively. This is consistent with the fact that constrained haemoglobin is always all slowly reacting in stopped-flow experiments²¹. The flash data are explained by assuming that the equilibrium between the dimers is completely in favour of $\alpha_1\beta_1$ in dilute salt at neutral pH, but is towards $\alpha_1\beta_2$ in high salt and acetate.

We now have to examine whether the first order step arises from a rate limiting dissociation reaction 8 of $\alpha_1\beta_1$ to single chains prior to reassociation to $\alpha_1\beta_2$:



Because the dimer-monomer equilibrium constant, $K_{2,1}$, in 0.09 M NaCl (pH 7.0) is not greater than $6.2 \times 10^{-9} \text{ M haem}$ (unpublished results of G. L. K.), and because the association rate constant of single chains in comparable conditions³² is approximately $8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, the minimum t_1 for reaction 8 is approximately 140 s. This value may

well be several orders of magnitude less than the real one, for hybridization data³³ suggest that $K_{2,1}$ is probably substantially less than 6.2×10^{-9} M haem. An $\alpha_1\beta_2$ model cannot therefore account for the observed t_1 of 3.5 s.

Consequences of the postulate that $\alpha_1\beta_1$ and $\alpha_1\beta_2$ dimers may coexist in certain conditions in appreciable fractions are that the interactions across the $\alpha_1\beta_1$ and the $\alpha_1\beta_2$ contact surfaces respectively must be comparable in strength and appreciable amounts of monomer must be formed. The monomer is a fast reacting species so that the percentage of "rapidly reacting haemoglobin" should be greater than the percentage of dimer. This is not observed. Furthermore, molecular weight studies in acetate^{18,19} reveal no molecular weights below the dimer value.

We conclude therefore that the data are better explained in terms of one kind of dimer, $\alpha_1\beta_1$, which may undergo a slow conformational change and conversion to constrained tetramer on ligand removal.

No Unliganded Dimer

The observed conformation change of the dimer was induced by ligand removal from both sites and does not provide any evidence about its cooperativity. It is, however, possible to predict the percentage of dimer in 0.09 M NaCl by comparison of the "drift" phase amplitudes in 0.09 M NaCl and 1 M NaI. Because both sets of conditions start from unconstrained $\alpha_1\beta_1$ dimers, such a prediction implies that the final unliganded, constrained equilibrium states must be spectrally equivalent. This situation requires either that the constrained dimer and tetramer have equivalent conformations, and are therefore presumably similar in cooperative binding properties, or that the constrained tetramer is not dissociated appreciably in either salt condition. This interpretation is also required by the stoichiometric titration of unliganded haemoglobin showing linear uptake of carbon monoxide³⁴.

It seems that the decision between the two alternatives must rest on molecular weight studies on unliganded haemoglobin which are fraught with technical difficulties. The present kinetic analysis, however, does not assume nor require any knowledge of the tetramer-dimer dissociation-association properties of constrained, unliganded haemoglobin. Recently, an extensive investigation of these properties (unpublished results of G. L. K.), in which a number of technical problems have been identified, has led to the finding that at concentrations as low as 6.2×10^{-7} M haem, the molecular weights at neutral pH in 0.09 M NaCl, 2 M NaCl and 1 M NaI are all greater than 60,000 daltons. Because these conditions vary greatly in their ability to dissociate oxyhaemoglobin, it has been concluded that there is no appreciable dissociation of constrained, unliganded tetramer within this concentration range. These results afford an explanation of why the value of n should be apparently independent of the state of aggregation of oxyhaemoglobin. In a wide range of conditions used to determine oxygen equilibrium curves, even in high salt concentrations, the unliganded, constrained tetramer would be undissociated. All cooperative effects could then take place in the tetramer which would dissociate to dimers only after the reaction with ligand had occurred and the structure of the tetramer changed to that of the liganded form. X-ray analysis⁴ shows that the subunits in the unliganded, constrained haemoglobin tetramer are strongly cross-linked by the C-terminal residues; these make the tetramer very stable. In this case, even if the dimer is completely non-cooperative (and the conformational change observed is associated with the Bohr effect^{35,36}) no rapidly reacting component of unliganded haemoglobin will be seen in stopped-flow experiments since it is effectively all tetrameric.

The present data clearly show that in 0.09 M NaCl (pH 7.0), the t_1 for the conformational change of the tetramer (probably not greater than 100 μ s) is a factor

at least 3.5×10^4 faster than that for the dimer (3.5 s). This information is of particular importance in assessing the relative efficiencies of the two major subunit contacts within the tetramer, $\alpha_1\beta_1$ and $\alpha_1\beta_2$, in transmitting the interactions which are the basis of cooperativity. Because the type of dimer which would exist in 0.09 M NaCl (pH 7.0) is believed to be $\alpha_1\beta_1$, our findings suggest that the $\alpha_1\beta_1$ contact region by itself is kinetically incompetent in transmitting interaction changes induced as a result of ligand removal (or probably binding). Although it is not possible to deny that a combination of both $\alpha_1\beta_1$ and $\alpha_1\beta_2$ contacts might be responsible for the very fast tetramer conformational change, it seems probable that the major contributory factor is due to a particularly efficient transmission of interactions (including terminal residues) across the $\alpha_1\beta_2$ contact surfaces. This interpretation is consistent with the structural findings of the X-ray investigations which show that the $\alpha_1\beta_1$ contact surfaces are intertwined and hardly show any movement during ligand binding and release; by contrast the $\alpha_1\beta_2$ contacts show extensive movement and display side chain amino-acid distortion (unpublished observations of H. Muirhead and J. Greer).

Some caution must be expressed concerning the simple analysis presented here, for the formation of tetramer hybrids of the type DD* has not been considered. These hybrids would also probably change conformation extremely quickly so that an additional rate limiting process of the type



would have to be considered in the analysis. It does not seem to be within our present experimental resolution, however, to distinguish contributory reactions of this type from the simple second order reaction (2). We have presented the simplest possible scheme consistent with the data, one which may well be subject to considerable refinements. The important feature, however, is that the conversion of rapidly reacting dimer to slowly reacting tetramer occurs by processes which are usually slow compared to ligand reactions. The fast dimer is slow.

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Growth Rate *in vivo* of Platelet Thrombi, produced by Iontophoresis of ADP, as a Function of Mean Blood Flow Velocity

by

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The rate of growth of a platelet thrombus is characterized by a first order rate constant. Its measurement will be most useful in future investigations of the mechanism of platelet thrombogenesis and its reactions to drugs.

In the normal circulation, platelets have no tendency to adhere to the walls of the blood vessels, to other blood cells or to each other. When a vessel is injured in any way platelets adhere to and collect on the damaged wall with great rapidity¹. Physiologically this increase in the adhesiveness of platelets ensures normal haemostasis; pathologically it is a cause of acute thrombosis, particularly in larger arteries which are diseased but also in small arterioles which supply tissue grafts undergoing rejection².

Clearly the elucidation of the mechanism which causes this change in the platelets is interesting in itself and of great practical importance. Experiments utilizing a quantitative method for observing platelet aggregation *in vitro*^{3,4} have provided much evidence about its mechanism (for reviews see refs. 5 and 6); this can be summarized as follows. Mammalian platelets are aggregated by various agents which seem to act through the formation or release of adenosine diphosphate (ADP). The effect of ADP passes through three stages. First, the platelets rapidly change their shape from flat disks with smooth surfaces into more spherical forms with variously shaped protrusions^{7,8}; this is a consequence of a direct reaction of the platelet surface membrane with ADP and seems to require no other factors⁹. It is rapidly followed by the adhesion of platelets to each other in loose aggregates¹⁰; for this, at least, fibrinogen and calcium are also required^{11,12}. Lastly, the platelets release the contents of certain cytoplasmic granules into the plasma^{13,14}. The released material includes ADP, thereby increasing its concentration in the plasma and providing the condition for the growth of platelet aggregates through a chain reaction¹⁵. During this release reaction the aggregated platelets become much more closely packed¹⁶. The evidence so far suggests that in the loose aggregates the platelets are bound to each other by long protein or glycoprotein molecules, probably fibrinogen, and that the binding forces between these molecules and the surface of the platelets are ionic, presumably via calcium⁵.

Whether the mechanism of platelet aggregation observed *in vitro* accounts for the formation of platelet thrombi *in vivo* is unknown. It is easy to cause platelets to adhere and aggregate in living vessels by injuring them in many different ways, ranging from the macroscopic mechanical damage caused by a cut to the microscopic thermal damage caused by a laser beam. The rate at which intravascular platelet aggregates embolize from such lesions can be used to measure thrombogenic activity¹⁷

and to find out whether substances which inhibit platelet aggregation *in vitro* can do so also *in vivo*¹⁸. But analysis of the thrombogenic process requires the initiation of the increase in platelet adhesiveness *in vivo* without damage to the vessel, indeed, with the least possible disturbance to its endothelial lining, and the quantitative determination of how the initiation and growth of the platelet aggregates depends on the following variables: (1) The cellular and geometrical properties of the blood vessel at the site of the thrombogenic stimulus; (2) the nature, duration and strength of that stimulus; (3) the concentrations in the blood of other factors, that is, fibrinogen and calcium, known to be necessary for platelet aggregation *in vitro*; (4) the concentration, distribution and reactivity of platelets in the flowing blood, and (5) the properties of the blood flow, particularly its velocity.

We wish to report the first part of an investigation into

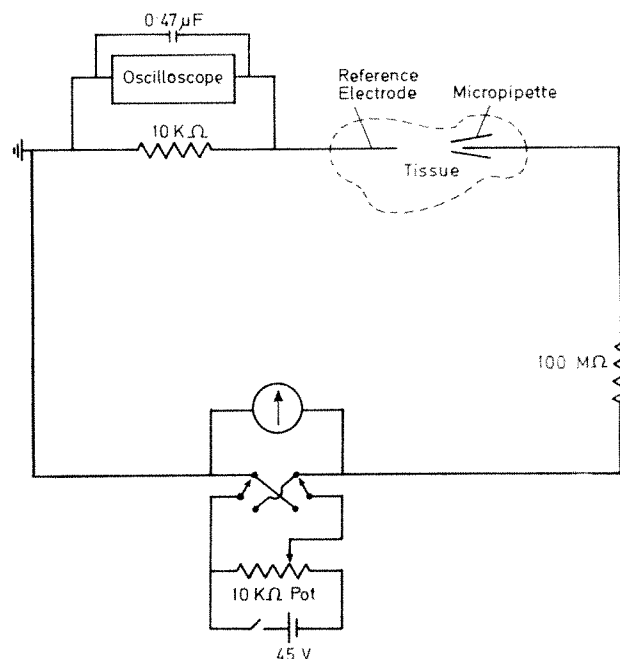


Fig. 1. Diagram of the circuit for the iontophoresis of ADP from micropipettes.

the formation of platelet thrombi or "white bodies" *in vivo* in conditions in which these variables were, as far as possible, measured or controlled, as follows. (1) The hamster cheek pouch was prepared for microscopic observation of uninjured venules of selected size. (2) The thrombogenic stimulus, ADP, was applied to the outside of the venules in minute, measured quantities by iontophoresis through micropipettes. (3) The concentrations of fibrinogen and calcium in the blood of different hamsters were assumed to be sufficiently supramaximal, as in other normal animals, not to affect the process. (4) The concentration of platelets was determined in the blood of twelve animals which were subsequently used for thrombogenic experiments. The concentrations were remarkably similar— $4.29 \pm 0.09 \times 10^8$ platelets/ml. (mean \pm s.e.m.). The reactivity of the platelets was also assumed to be similar in each animal. The quantitative distribution of the platelets in the blood stream across the venules could not yet be determined and remained unknown; possibly it could vary with changes in the velocity of the blood flow. (5) The mean velocity of the blood flow was measured in the venules by a simple technique. Results show that, in the conditions of these experiments, the rate of growth of platelet thrombi depends markedly on this velocity.

Iontophoretic Application of ADP

The application of drugs to the microcirculation by iontophoresis through micropipettes was introduced to measure the reactivity of different types of small vessels¹⁹. We used the method to apply ADP to the vessels. Micropipettes measuring 1–3 μ m across the tip were filled²⁰ with a 1×10^{-2} M solution of the sodium salt of ADP in distilled water. The solution in the micropipette was connected by an external circuit (Fig. 1) to a silver-silver chloride reference electrode. The iontophoretic movement of ADP out of the micropipettes was measured by a radioactive technique which will be described in detail elsewhere²¹. Micropipettes were filled with 10^{-2} M tritiated ADP of the highest available specific activity—44.3 Ci/mole. The tip of such a micropipette and the reference electrode were immersed in a small volume of saline. When a negative potential was applied to the pipette, radioactivity appeared in the saline and increased linearly with time for at least 20 min. With a current of 300 nA, ADP was expelled from the micropipette at a rate of about 2×10^{-14} mole/s, corresponding to about 1×10^{10} molecules ADP/s. The technique thus provided a measure



Fig. 3. Electron micrograph of the site in a venule to which ADP was applied iontophoretically by a current of 300 nA for 50 s. The lumen contains red cells (R) and a loose aggregate of platelets (Pl), some of which are adhering to an endothelial cell (E). The cytoplasm of this cell is less electron-dense than that of a neighbouring pericyte (P) and the cytoplasm of endothelial cells, not shown, at some distance from the site. A neutrophil granulocyte (NGR) lies outside the endothelial cell. ($\times 8,400$)

of the minute quantities of ADP which could be applied iontophoretically to small blood vessels and a means for controlling these quantities by varying the strength and duration of the electric current. 300 nA was the current used in the experiments to be described.

White Bodies and Thrombogenic Site

Golden hamsters of both sexes, weighing about 100 g, were anaesthetized with intraperitoneal pentobarbital (6 mg/100 g body weight). The cheek pouch was everted and spread on a special 'Perspex' stage (see Fig. 1 from ref. 19). The top layer and fine connective tissue were removed and a Leitz Laborlux microscope (mostly with a $\times 20$ objective and a $\times 10$ eyepiece) was focused on the thin lower layer which was transilluminated from below with light from a xenon lamp. The preparation was bathed continuously in an aerated saline solution (composition in mM: NaCl 131, KCl 4.0, CaCl_2 3.0, MgSO_4 1.0, glucose 10 and Tris 5.0; pH 7.4), and maintained accurately at 37°C. The preparation remained in good condition, as assessed by the blood flow in and the reactivity of the vessels and by the absence of oedema, for at least 2–3 h.

A micropipette filled with ADP solution was manipulated closely against the outside of a small venule (diameter 40–70 μ m). The reference electrode was immersed in the bathing solution. When a negative potential was applied to the micropipette to eject ADP, the diameter of the venule was unaltered (Fig. 2). The application of ADP for more than 5 s caused the formation of a white body attached to the inside of the venule beneath or slightly downstream from the tip of the pipette (Fig. 2). A similar application of ADP to an arteriole caused it to constrict. White bodies formed in most arterioles but in all venules; for this reason venules were chosen for making measurements on the growth of white bodies. The iontophoretic application of nucleotides closely related to ADP, including AMP and GDP at similar

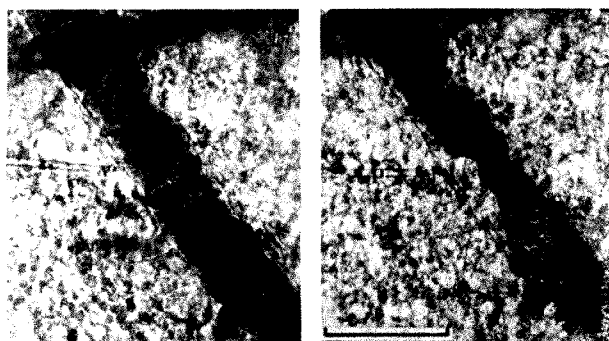


Fig. 2. Normal venule before (left) and after (right) the application of a negative potential to the microelectrode shown which was filled with 10^{-2} M ADP. The iontophoretic current was 150 nA. The photograph on the right was taken when the white body had grown for 50 s. Scale, 100 μ m.

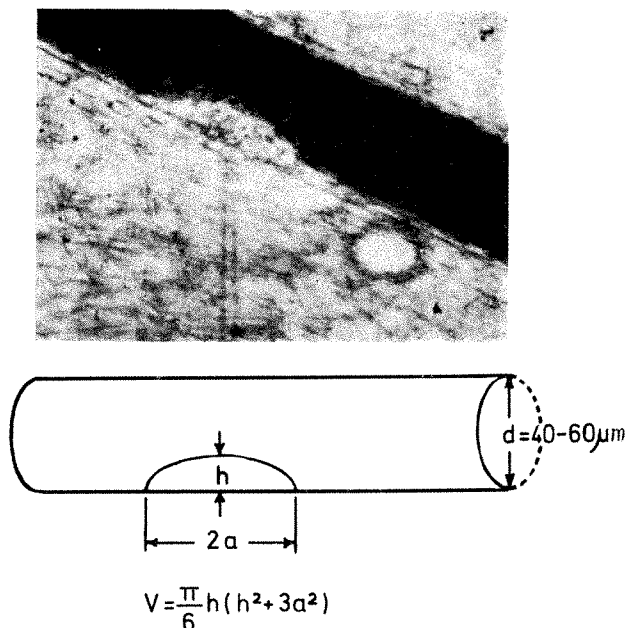


Fig. 4. Growing white body produced by the iontophoretic application of ADP to a venule. The white body appears as a segment of a circle. Below is a diagram showing the measurements made for calculating the volume of the white body by the formula.

concentrations, did not produce white bodies in the venules, nor did inorganic phosphate or saline. The effect of ATP, which *in vitro* inhibits the aggregating effect of ADP (ref. 4), was variable, presumably because of breakdown to ADP. These results indicated that the white body-producing effect was specific for ADP.

The next problem was to establish whether the site where the iontophoretic application of ADP caused white bodies to form was normal or abnormal, and, if so, to find some measure of the abnormality. Cortical blood vessels of anaesthetized rabbits can be injured so slightly that no white bodies form in them unless ADP is also applied²², and this suggests that the formation of white bodies depends on the additive effects of mechanical injury and ADP. In our

experiments it was possible that the dissection of the pouch and/or the iontophoretic current could have caused sufficient injury to the venules for ADP to have a similar additive effect. To assess this, serial electron micrographs were made of the sites to which either ADP, or GDP as a non-thrombogenic control substance, had been applied iontophoretically; the preparations were immediately fixed in glutaraldehyde followed by osmic acid²³. The pictures showed that the plasma membrane of the endothelial cells and the junctions between them were intact and apparently normal; the only abnormality was some loss of electron density of the endothelial cytoplasm compared with that of cells in control areas (Fig. 3). This abnormality occurred, however, with both ADP and GDP and was therefore not associated specifically with the thrombogenic action of ADP. The cause of the abnormality is still uncertain.

White bodies have been produced by damaging small blood vessels electrically²⁴⁻²⁶; however, the currents required for this were up to 300 μ A rather than 300 nA, that is, a thousand times greater than the iontophoretic current used in our experiments. Furthermore, any heat produced would, in the conditions of these experiments, be rapidly dissipated by the superfusing saline as well as by the blood stream in the vessels. It was concluded that the iontophoretic applications of ADP as described did not cause any clear or specific abnormality to the vascular endothelium but that some mild, unspecific change did occur inside the cells; this has to be further investigated.

Growth of White Bodies

With continuing passage of the iontophoretic current each white body continued to grow for about 1 min. Thereafter, the white bodies embolized in whole or in part and new ones continued to form and embolize at the same site until the current was switched off. The rate of growth of white bodies was determined by filming the site where ADP was applied at 32 f.p.s. for 2 s every 5 or 10 s until the white body embolized. As long as the white bodies remained small relative to the diameter of the venules, their outline in the lumen approximated to a segment of a circle (Fig. 4). The base ($2a$) and height (h) of each growing white body after increasing time were measured on the film. On the assumption that the shape of the white body approximated to a segment of a sphere

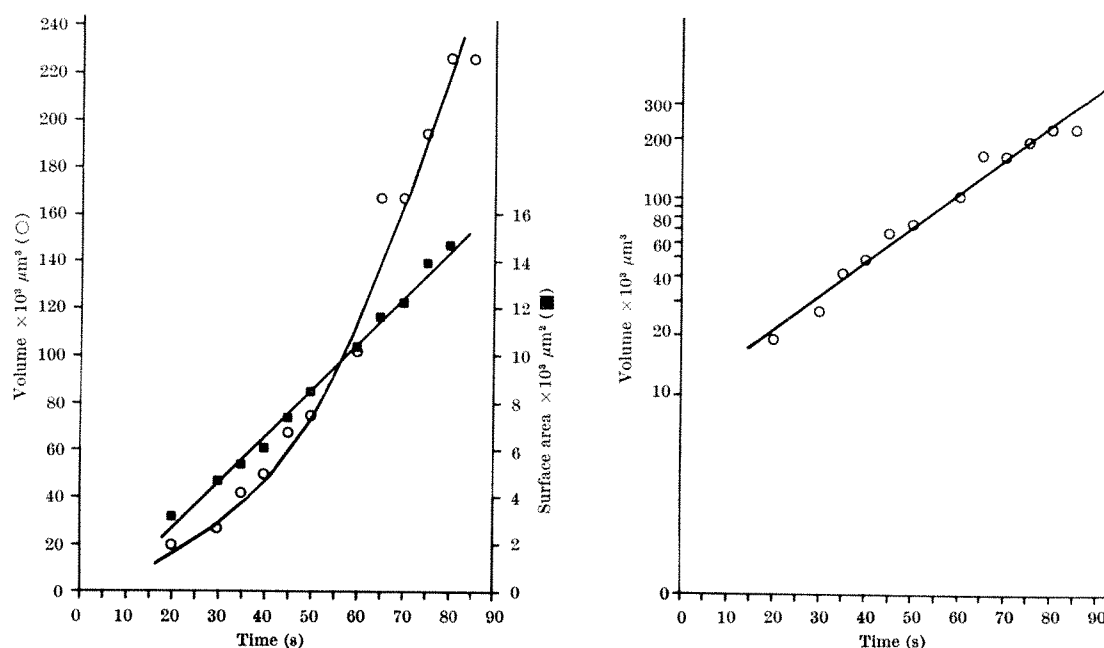


Fig. 5. Increases in surface area (■) and volume (○) of a white body with time. On the right, the increase in volume is plotted semi-logarithmically against time, showing the line of best fit.

(radius r), its volume (V) and surface area (A) were calculated by $A = 2\pi rh$ and $V = \frac{1}{3}\pi h(h^2 + 3a^2)$. Clearly this assumption was only a reasonable approximation; for one thing, it neglected the effect of the curvature of the vessel wall. This source of error was minimized by using for the measurements only white bodies with heights less than one-third of the vessel diameter.

The surface area of the white bodies increased linearly with time while the volume increased exponentially (Fig. 5). Therefore a semi-logarithmic plot of volume against time provided a rate constant of the growth of the white body, which itself provided a measure of the growth rate and a means for determining the effect of the different variables on it.

Growth of White Bodies depends on Blood Flow

The mean blood flow velocity in venules in which white bodies were produced was measured by a new, simple technique. A white body was produced in the usual way about 500 μm upstream from the site where the growth rate of another white body had just been determined. When the upstream white body was still small but clearly visible, the iontophoretic current was switched off; within

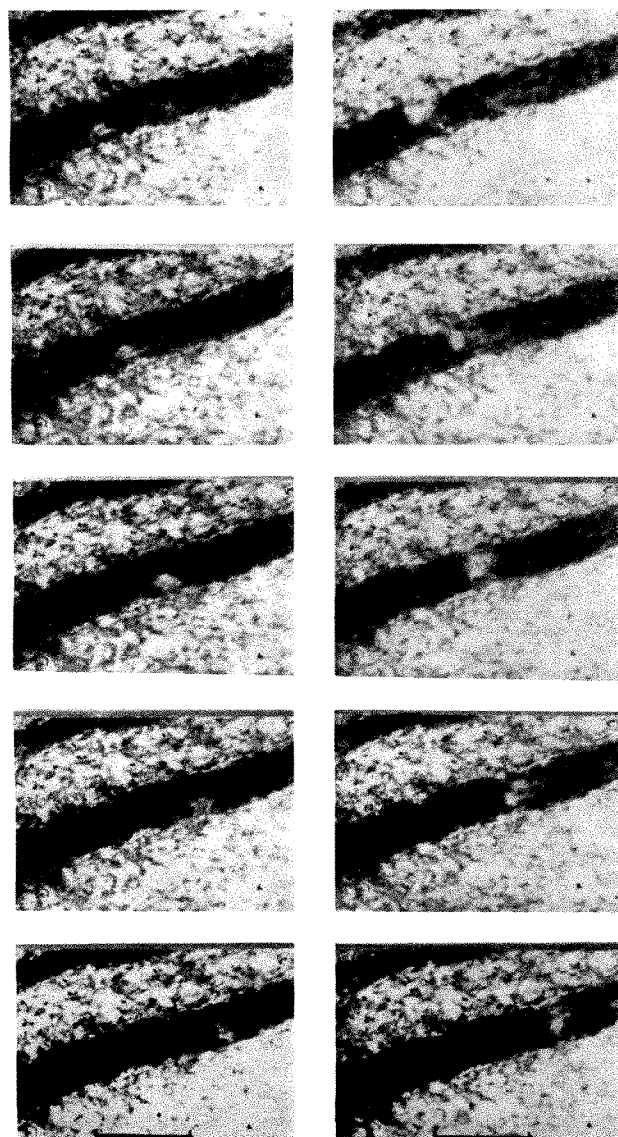


Fig. 6. Serial photomicrographs, at intervals of $1/16$ s, of two white bodies passing along the same venule. The white body on the left moved at $625 \mu\text{m/s}$ and that on the right at $655 \mu\text{m/s}$. Scales, $100 \mu\text{m}$.

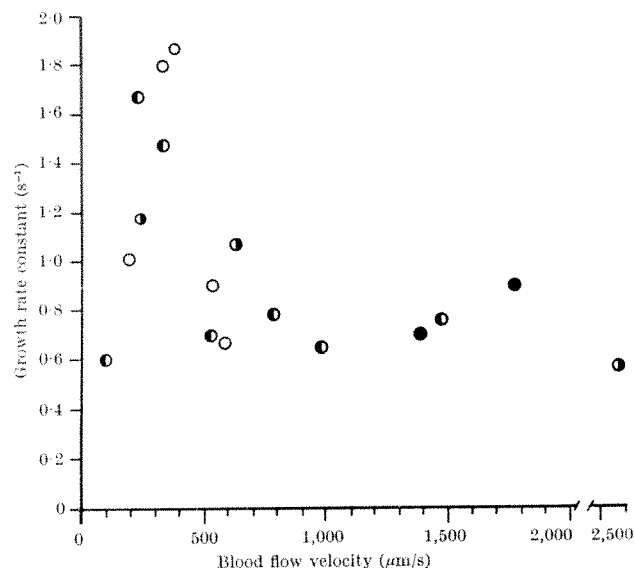


Fig. 7. Effect of mean blood flow velocity in venules on the growth rate constant (s^{-1}) of white bodies: results from seventeen experiments. Diameter of the venules: \circ , 49–49 μm ; \bullet , 50–59 μm ; \bullet , 60–69 μm ; \bullet , > 70 μm .

a few seconds the white body embolized. With the microscope focused on the downstream site, the embolus was filmed at 32 f.p.s. as it was swept along by the blood stream. The film was used for measuring the velocity at which the white body moved. An example is shown in Fig. 6: the velocities of two different white bodies were measured in the same vessel, with very similar results—625 and 655 $\mu\text{m/s}$. In three other such experiments, the velocities of different white bodies in the same vessel were equally similar although carried at different distances from the vessel wall. The results of blood flow velocity measurements made in this way were also very similar to measurements made in such venules in the hamster cheek pouch with a particle velocity meter²⁷.

In seventeen experiments, measurements were made of both mean blood flow velocity and the growth rate constant of white bodies, produced in venules of similar size by iontophoretic currents of 300 nA (Fig. 7). The growth rate of white bodies increased with increasing mean blood flow velocity up to a sharp maximum at velocities of 300–400 $\mu\text{m/s}$. With higher velocities the growth rate decreased and remained approximately constant, at about half the maximum, as the mean flow velocities increased from about 600 to about 2,500 $\mu\text{m/s}$. With velocities greater than 3,000 $\mu\text{m/s}$, no white bodies formed.

The causes of this relationship have not yet been established. It is reasonable to suggest, however, that the rate of growth of white bodies in the conditions described was determined by at least two opposing factors—the rate at which platelets were brought to the site and the rate of shear which would tend to prevent platelets from adhering. Both factors depend on blood flow velocity. Our observations suggest that when the mean blood flow velocity was less than about 400 $\mu\text{m/s}$, the increasing growth rate of the white body was a result of the increasing rate of supply of platelets which were able to adhere and that, at higher flow velocities, shear increased to such an extent that it prevented more platelets from adhering.

This interpretation is based on assumptions the validity of which still has to be determined. First, it is assumed that the velocity profile in the venules was not parabolic but rather flat, almost to the walls, so that measurements of blood flow velocity indeed represented mean velocity. The initiation of white bodies presumably depends primarily on the concentration and the movement of platelets in immediate proximity to the vessel wall, where flow velocity is known to be slow. As a white body grows

into the lumen, the mean blood flow velocity past it must increase if the pressure remains constant, and this local increase in flow velocity presumably also affects the process. To investigate these flow effects we are now measuring velocities of blood cells flowing along the vessel wall by means of high speed cinematography.

Another assumption is that when platelets make contact with the thrombogenic vessel wall or with other platelets in the growing white body, the time of contact is long enough, whatever the rates of flow and shear, for the change in platelet shape to occur which is a prerequisite for aggregation by ADP *in vitro*⁹. It will be necessary to find means of measuring contact times at different shear rates.

Finally, the demonstration that the growth rate of a platelet thrombus is characterized by a first order rate constant will be very useful in the further investigation of the mechanism of platelet thrombogenesis and in measuring the *in vivo* effectiveness of drugs capable of inhibiting platelet aggregation *in vitro*.

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Geomagnetic Polarity Change, Volcanic Maxima and Faunal Extinction in the South Pacific

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Studies of deep-sea sedimentary cores from Antarctic Pacific waters show that some volcanic maxima occurred when the geomagnetic polarity was changing. Upper mantle activity and geomagnetic polarity change may therefore be related. Coincidences of faunal extinction and geomagnetic polarity change may be explained by corresponding volcanically induced climatic changes.

GEOMAGNETIC field reversals occurred more than twenty times during the past 4 m.y. (ref. 1) and probably more than one hundred times during the Tertiary². The most significant aspect of this discovery is the recognition of important features of the history of geomagnetic polarity¹ in the linear magnetic anomalies of the oceanic crust^{3,4}, and their convincing interpretation in terms of sea-floor spreading⁵⁻⁸.

The geomagnetic polarity history has also been recognized in deep-sea sedimentary cores^{9,10}. In such studies, several microfaunal extinctions and appearances have been recognized as synchronous, and virtually simultaneous with the period of transition between opposite geomagnetic polarities⁹⁻¹³ which lasts about 5,000 years¹⁴. The reasons for these extinctions and appearances are unknown, but they may be the result of climatic change and polarity change being related^{15,16}, or of increased mutation rates during polarity changes¹⁷, although several authors^{15,18,19} oppose this possibility.

Heirtzler²⁰ has recently speculated on a relationship between earthquake activity (and by implication upper mantle activity) and geomagnetic polarity change. He reasons that because there is evidence to show that earthquakes of magnitude 7.5 or greater may cause wobble of the spin axis²¹, it is therefore conceivable that an Earth wobble may be of a magnitude sufficient also to cause reversal of the geomagnetic field. Here we present data pertinent to Heirtzler's speculations²⁰, and we expand such speculations to include our preferred explanation of the synchronous geomagnetic polarity changes and faunal extinctions.

We know of no method to detect the time of occurrence and frequency of large earthquakes in the geological past. Stress release is often manifested in volcanic activity, however, so a search may be profitably made for evidence of relationships between volcanic maxima and geomagnetic polarity change. Volcanism certainly may have more than one cause, and on a global scale must be virtually continuous, so we therefore emphasize our use of the term "volcanic maxima". If relationships exist between polarity change and upper mantle activity they are more likely to be found in oceanic areas, for there the mantle is most mobile and accessible, and (in all probability) more commonly at "threshold", in terms of potential volcanism. Our search therefore examines the character of volcanic activity during polarity change in some deep-sea sedimentary cores.

The cores used were collected during cruises of USNS Eltanin and their locations are included in Fig. 1 and Table 1. Core selection was based only on length, minimum age, and geographic position. Collection, transportation, and sampling procedures have been described elsewhere²². In this study, specimens for palaeomagnetic study were taken at 10 cm intervals, demagnetized at 150 oersteds using a four axis tumbler²³ and measured using a 5 Hz spinner magnetometer²⁴.

At 20 to 30 cm intervals the palaeomagnetic specimens were then weighed dry, disaggregated and washed on a 63 μ m mesh Tyler screen. The sand-size fraction was examined to determine the relative abundance of volcanic glass shards and the nature of the micro-fossils. Restriction to the sand-sized fraction probably eliminates much

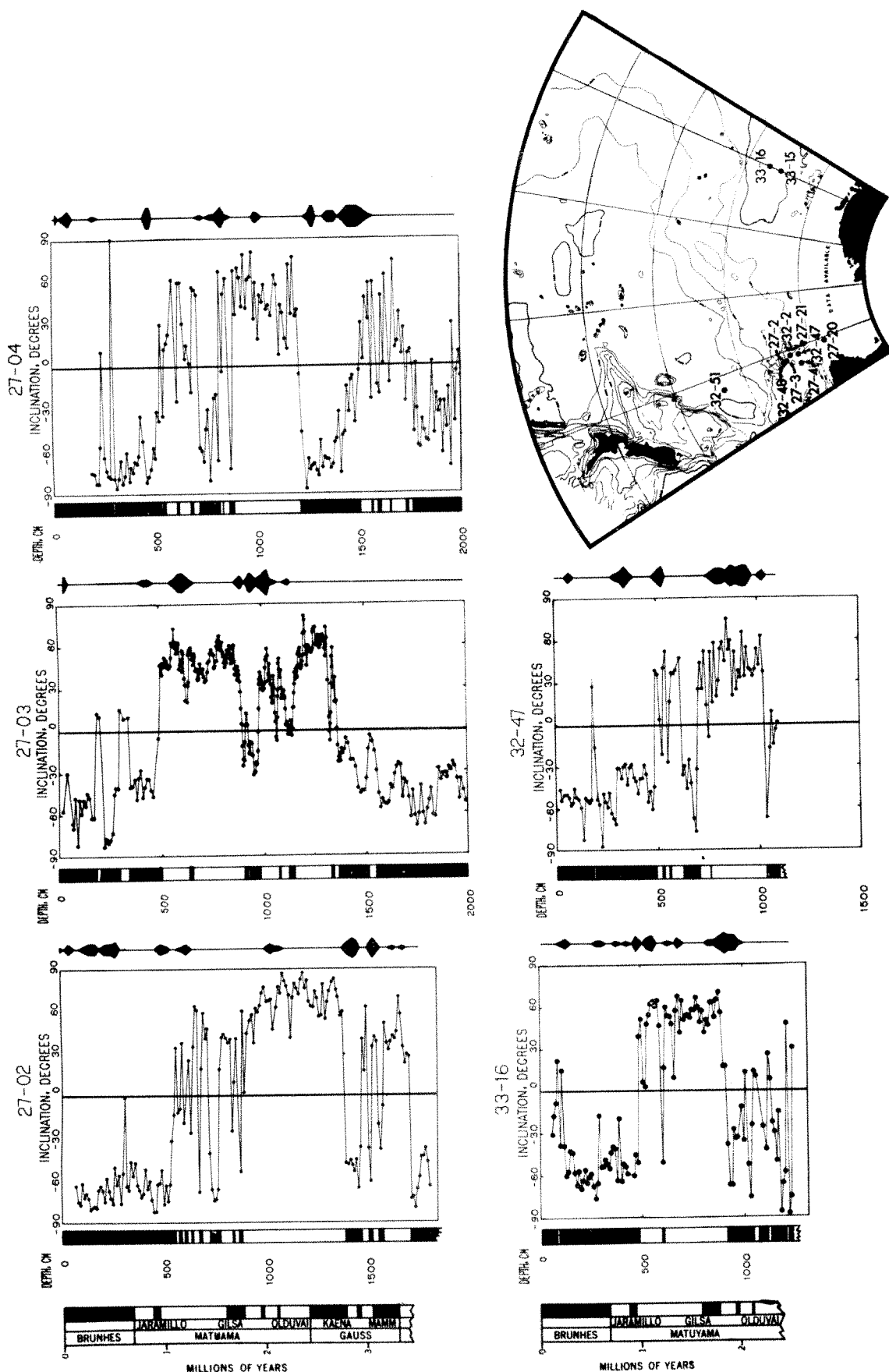


Fig. 1. Inclination of natural remanent magnetism, and fraction of sand-sized ash in five selected cores. Location of these and other cores (examined in Fig. 2) is given in the inset, and in Table 1 (core number at head of each plot). In each core, the inclination of natural remanent magnetism is shown for each specimen. Negative inclination (above horizontal) is normal polarity. Inclination without sign is positive (below horizontal) or reversed polarity. Depth of specimen in core shown next to each plot, where a polarity log is also shown; this is a non-subjective plot of the inclination data, with black = normal polarity; clear = reversed polarity. The polarity time scale at the left of each plot, from left to right, of the age in m.y.; the names of the polarity epochs; the names of the polarity events; and the polarity history. The histogram to the right of each log expresses the fraction of sand-sized volcanic glass shards and other volcanic material in the given specimen. It results from examination using a binocular microscope and is defined using micropaleontological conventions. The x-axis definition is five-fold as follows: width 4 (width) = very abundant; 3 = abundant; 2 = common; 1 = uncommon; 0 (fine line) = very rare or absent. Note that the inclination plot of core 27-03 includes a sequence of data from specimens taken at 2 cm intervals for a special study of the Matuyama epoch polarity history and subjected to a five-point running average. The corresponding polarity log is a slightly subjective interpretation of these data.

Table 1. DETAILS OF CORE LOCATION AND STATISTICAL ANALYSIS OF THE RELATIONSHIP BETWEEN MAGNETIC POLARITY BOUNDARIES AND VOLCANIC ASH HORIZONS

Core No.	Latitude (degrees south)	Longitude (degrees east)	Water depth (m)	Core length (m)	Percentage ash (<i>Pa</i>)	No. of reversals	No. of coincidences	<i>Pa.g</i>
E27-02	63° 00'	177° 42'	3,358	16.97	42.6	8	4	0.469
E27-03	66° 04'	176° 30'	3,520	21.66	34.1	8	6	0.023
E27-04	68° 03'	174° 35'	3,433	19.89	37.3	8	5	0.137
E27-20	71° 57'	178° 36'	2,116	5.20	—	—	—	—
E27-21	69° 02'	179° 50'	3,440	16.07	34.1	8	3	0.572
E32-02	67° 22'	178° 44'	3,656	10.21	67.1	3	3	0.303
E32-47	68° 06'	176° 10'	3,413	10.93	45.4	5	5	0.019
E32-51	58° 50'	176° 52'	4,845	7.53	—	—	—	—
E33-15	65° 07'	199° 45'	4,823	11.52	21.6	8	5	0.158
E33-16	63° 14'	199° 59'	4,890	15.03	67.3	8	6	0.489

Percentage ash is based on Fig. 1: it is the fraction of the core in which any sand-sized ash is found, and is not a relative volume count. It follows that this is also the probability (*Pa*) of finding sand-sized ash in any part of each core. Number of reversals is the number of known polarity changes during the last 2.5 m.y., or less if the given core does not reach that age. Number of coincidences is the number of times that an ash occurrence is found at a polarity change in Fig. 2. A ± 30 cm range is allowed for detection of such coincidences, because reworking and polarity recording imperfections are likely to be present. *Pa.g* is the probability that the observed number of coincidences of ash and geomagnetic polarity change are simply the result of coincidence of two randomly occurring phenomena. No analysis is given for E32-51: this is illustrated in Fig. 2 (right) and is not amenable to analyses.

very fine volcanic material, which simplifies the search for evidence of regional volcanic maxima.

Sediment type in the cores varies greatly. It includes lutite, silicious and carbonate ooze, and glacial-marine material. Most cores lack planktonic foraminifera, because of their solution at the depth of the cores, but when present they are restricted to one species, *Globigerina pachyderma*. Radiolaria are much more diverse, and are often abundant throughout the cores, and frequently offer the only micropalaeontological control possible.

The palaeomagnetic results are shown in part in Fig. 1 and completely in Fig. 2. Material as old as the Gilbert Epoch is identified. The correlation lines for the major polarity (epoch) boundaries have been readily confirmed by applying the radiolarian zonation established by Opdyke *et al.*⁹ and Hays and Opdyke¹³. The species which were particularly valuable in our correlations were *Prunopyle titan* and *Desmospyris spongiosa* (the extinction of which coincides approximately with the Gauss-Matuyama boundary); and (in northern cores) *Eucyrti-*

dium calvertense whose extinction is, we believe, associated with the Gilsa event¹ and *Saturnulus planetes*, which disappeared near the Brunhes-Matuyama boundary (0.7 m.y.). Correlation of the shorter duration magnetic polarity events is shown in Fig. 2 only for the events in the Matuyama epoch. Palaeontological controls for these are simply not available locally, and the correlations are therefore made subjectively using the magnetic data alone and, where these are not clear, by extrapolation of sedimentation rates. Question marks are added, where appropriate, to Fig. 2.

Volcanic shards, which usually do not form megascopically distinct layers, were found in all the sedimentary cores examined for this study. Within each core large variations in the relative abundance of the shards occur, ranging from complete dominance to virtual absence (Figs. 1 and 2). The glass shards occur mostly as flaky fragments, very similar to those illustrated by Ninkovitch^{25,26}. The data clearly show periods of volcanic maxima. A general increase in the size of the glass shards, and in the amount of other volcanic debris, such as small volcanic pellets, occurs in cores nearer the Balleny Islands, suggesting that the source is at least in part in this vicinity. Similar but finer volcanic glass is found at the same palaeomagnetic horizon in the central Pacific (core E33-16, Fig. 1) strongly suggesting either contemporaneous activity in the area, or (more likely) a very large fallout area, possibly larger than previous considerations allow²⁷.

Figs. 1 and 2 clearly show that when geomagnetic polarity changes were taking place, volcanic maxima were also occurring, at least locally. Volcanic fragments are most abundant and consistent in their occurrence close to the Brunhes-Matuyama boundary (0.7 m.y.), near the Matuyama-Gauss boundary (2.4 m.y.), and during at least two periods within the Brunhes epoch (0 to 0.7 m.y.). Other less well defined horizons are related in part to the sediments of the shorter duration events, and the Gauss-Gilbert boundary. Volcanism was clearly taking place locally during other periods. It is intriguing that the Brunhes-Matuyama and Matuyama-Gauss polarity changes are remote from evidence of

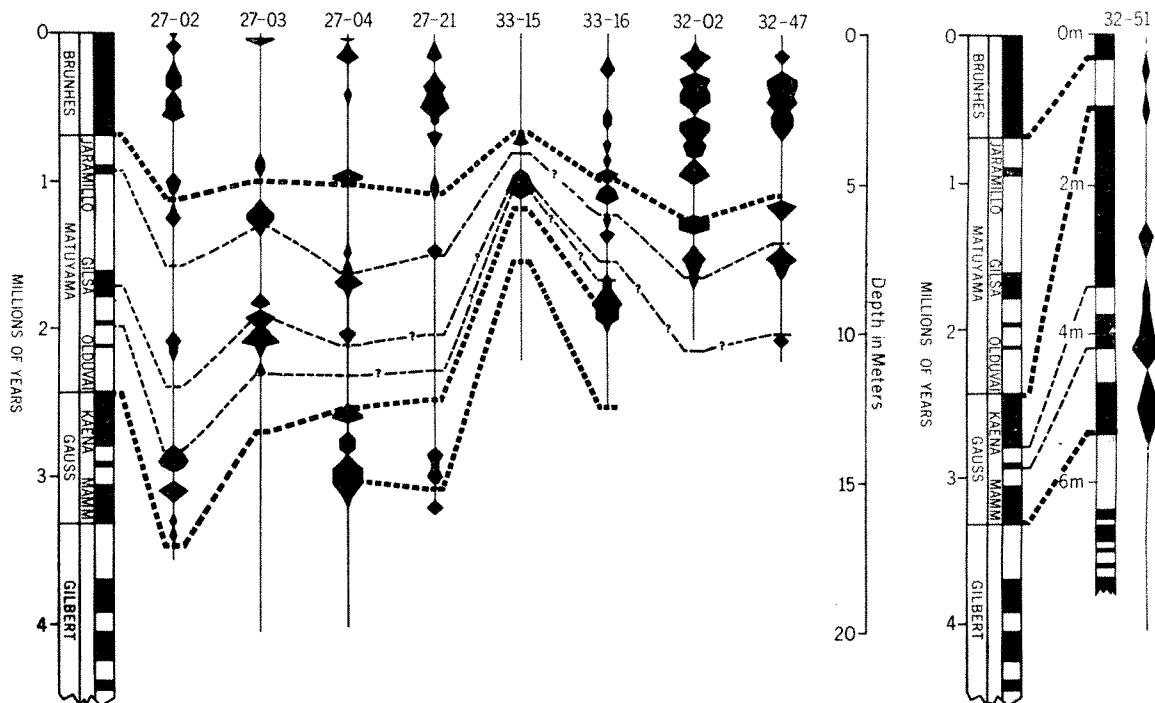


Fig. 2. Histograms of volcanic material fraction in each core and the geomagnetic correlation lines. For description of the volcanic fraction histogram construction see legend to Fig. 1. Geomagnetic polarity time scale after Cox¹ as in Fig. 1. The correlations at the three magnetic epoch boundaries are given by heavy dashed lines. These are confirmed micropalaeontologically. The correlations of the polarity events within the Matuyama epoch are based on magnetic and sedimentation rate data only. Core 32-51 features a major discontinuity, which results in most of the upper 2.4 m.y. of the core being absent.

volcanic activity in only one of the fourteen such examples contained in the eight cores shown in Fig. 2 (left). Nevertheless, we obviously cannot dismiss the possibility that the results are simple coincidence. Despite obvious experimental and analytical difficulties, we therefore offer a statistical analysis (Table 1). Our approach is to restrict analysis to sediments aged 2.5 m.y. or less if the cores do not reach this age, as is the case in E32-02 and E32-47. We assume that eight polarity changes have taken place: one each for the Brunhes-Matuyama and Matuyama-Gauss boundaries and two each for the three known events in the Matuyama. Counting the percentage of the core in which any ash is observed (Fig. 2) provides the probability (Pa) of ash occurring in any part of the core. Combined with the number (N) of observed coincidences of ash and polarity change (Fig. 2 and Table 1) we can then express the probability ($Pa.g$) of the observed occurrences of simultaneous polarity change and ash deposition being a simple coincidence of two independent variables. Other statistical approaches are possible.

In cores E27-03 and E32-47 there is only a 0.02 probability that the ash and polarity change coincidences occur by chance only (Table 1). In cores E27-04 and E33-15 the probability is less than 0.16; but this rises to 0.30 in E32-02, where the ash content is very high. In cores E27-02 and E33-16, fewer coincidences and high ash content, respectively, reduce $Pa.g$ to almost even chance. Only in core 27-21 is $Pa.g$ greater than 0.50, meaning that in this core there is a better than even chance that the three observed coincidences of polarity change and ash are random events.

Fig. 2 (right) contains the results from one other core. E32-51 contains a disconformity, which has resulted in only a few centimetres of Brunhes sediment being above the oldest Matuyama, and what appears to be a complete Gauss section. Micropalaeontological evidence confirms these correlation lines. Both the Kaena and Mammoth events are clearly displayed: no additional events in the Gauss are indicated. Volcanic materials are at a maxima very close to these events and limits of the Gauss epoch.

Palaeomagnetic and potassium-argon analyses of lavas on Nunivak Island²⁸ (Fig. 3a) show that volcanic maxima on the island are most marked close to the geomagnetic polarity reversals defined. McDougall and Chamalaun's²⁹ results from the Indian Ocean islands of Mauritius, Réunion, and Rodriguez (Fig. 3b) show frequent volcanism during the Brunhes epoch and there may be a correlation between volcanic activity and polarity change during the preceding 3 m.y. A coincidence of ash layers and geomagnetic polarity change is included in the results of Ninkovitch²⁵ from study of a series of cores taken from east of New Zealand (Fig. 3c). Here five megascopically distinct tephra are reported. One is closely associated with the polarity change at the end of the Jaramillo event, two straddle the Brunhes-Matuyama polarity change, and two occur within the Brunhes. Similarly, Ninkovitch *et al.*³⁰ report megascopic ash layers in the North Pacific (core F20-119) which coincide with the Jaramillo event (Fig. 3d) and Genter *et al.*³¹ report an ash layer in the north-east Indian Ocean (core V19-153) which coincides with the Brunhes-Matuyama boundary. While we find these observed correlations of volcanism and polarity change difficult to accept as purely coincidental, we stress our awareness of the fact that many more data are required to prove that the implied relationship is meaningful.

We now briefly examine the relevance of our data to problems of climatic changes and microfaunal extinctions and appearances. If significant widespread increases in volcanism have occurred during restricted intervals of geological time, then climatic changes are very likely, particularly at high latitudes, because volcanic ash, which remains in the atmosphere for prolonged intervals of time and moves poleward at high altitudes, inhibits solar radiation at the surface^{32,33}. In this context it is therefore

possibly significant that a distinct world-wide cooling occurred near the Brunhes-Matuyama boundary^{13,34,36}, and that other climatic coolings may be associated with the Gilsa event and Matuyama-Gauss boundary³⁷. Increases in volcanism within the Brunhes epoch as indicated by us in the Southern Oceans occur in New Zealand (personal communication from H. W. Wellman). This coincides with, and may be related to, the much greater climatic fluctuation of the past 0.7 m.y., compared with the preceding million years^{34,36,38,39}.

Of eight observed microfaunal extinctions and appearances in the Southern Ocean, six occur either during or very close to geomagnetic polarity changes⁴⁰. We suggest that the climatic changes which can result from volcanic maxima are much more plausibly the cause of such extinctions and appearances than increased radiation at the water surface during any dipole collapse accompanying a polarity change, although other explanations must be considered.

We conclude that our results provide sufficient evidence to justify serious consideration of Heirtzler's²⁰ speculations of a connexion between geomagnetic polarity change and upper mantle activity, which was also proposed by Hide⁴¹. Volcanic maxima during polarity changes may be expected to have an influence on climatic conditions, particularly at very high latitudes, and may therefore also be the indirect cause of those microfaunal extinctions which have occurred during geomagnetic polarity changes.

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LETTERS TO NATURE

PHYSICAL SCIENCES

The Non-existence of Stationary Infinite Newtonian Universes and a Multi-dimensional Model of Shot Noise

HOLTSMARK¹, in an electrostatic context, and Chandrasekhar², in the case of a Newtonian gravitational field, have given a simple model which describes the electrostatic or gravitational field at an arbitrary point in space. They take the point as the origin of coordinates and calculate the field there due to point charges or masses which are scattered randomly and homogeneously throughout the space. Specifically, the field is

$$\mathbf{X}_R(0) = K \sum_{j=1}^N \mathbf{t}_j / |\mathbf{t}_j|^3 \quad (1)$$

where K is a proportionality constant, and in a sphere of radius R it is supposed that there are N points scattered randomly and located at $\mathbf{t}_1, \dots, \mathbf{t}_N$. By letting N and $R \rightarrow \infty$ in such a way that the density of points $3N/4\pi R^3 \rightarrow \lambda$, Holtsmark showed that the probability distribution of $\mathbf{X}_R(0)$ converges to a limit, being the distribution of $\mathbf{X}^p(0)$, say.

Now, if this model is to be at all realistic, it must have the property of stationarity. That is, if our point is taken not at the origin (which for equation (1) is the centre of the sphere R) but at \mathbf{t} , say, then by starting from

$$\mathbf{X}_R(\mathbf{t}) = K \sum_{j=1}^N (\mathbf{t}_j - \mathbf{t}) / |\mathbf{t}_j - \mathbf{t}|^3 \quad (2)$$

and proceeding to the limit with R and $N \rightarrow \infty$ as before, then the resulting distribution, of $\mathbf{X}^p(\mathbf{t})$, say, should be the same as for $\mathbf{X}^p(0)$. A mathematical analysis to be given elsewhere³ shows that this is not so; hence the non-existence statement in the title.

The point of this conclusion, which may at first sight seem to be a piece of mathematical pedantry, is more apparent when we consider simultaneously³ the field at two points separated by a distance $2\mathbf{t}$. The limiting distributions obtained by taking the limit R and $N \rightarrow \infty$ as before in the two possible definitions (equations (3) and (4) below) are different, yet comparison with equations (1) and (2) gives no reason for preferring either definition.

$$(\mathbf{X}_R(0), \mathbf{X}_R(2\mathbf{t})) = K \sum_{j=1}^N (\mathbf{t}_j / |\mathbf{t}_j|^3, (\mathbf{t}_j - 2\mathbf{t}) / |\mathbf{t}_j - 2\mathbf{t}|^3) \quad (3)$$

$$(\mathbf{X}_R(-\mathbf{t}), \mathbf{X}_R(\mathbf{t})) = K \sum_{j=1}^N ((\mathbf{t}_j + \mathbf{t}) / |\mathbf{t}_j + \mathbf{t}|^3, (\mathbf{t}_j - \mathbf{t}) / |\mathbf{t}_j - \mathbf{t}|^3) \quad (4)$$

The model here is a special case of a generalization of shot noise in which points are scattered homogeneously and randomly (as a Poisson process) in an n -dimensional Euclidean space with density λ , with any point \mathbf{t}_j contributing the disturbance $\mathbf{f}(\mathbf{t}_j - \mathbf{t})$ to the total disturbance (summed over j)

$$\mathbf{Y}(\mathbf{t}) = \sum \mathbf{f}(\mathbf{t}_j - \mathbf{t}) \quad (5)$$

at any point \mathbf{t} . [In equations (1) and (2), $n=3$ and $\mathbf{f}(\mathbf{t}) = K\mathbf{t}/|\mathbf{t}|^3$. If instead the Yukawa⁴ disturbance function $\mathbf{f}(\mathbf{t}) = K\mathbf{e}^{-at}(1 + at)\mathbf{t}/t^3$, where $t = |\mathbf{t}|$, is used, then the distributions of $\mathbf{X}^p(\mathbf{t})$ and $\mathbf{X}^p(0)$ are the same.] The existence of the limiting distributions for $\mathbf{X}^p(\mathbf{t})$ and $\mathbf{Y}(\mathbf{t})$ depends on the behaviour of $\mathbf{f}(\mathbf{t})$ for large $|\mathbf{t}|$, and possibly also (as with equation (1)) on the order of summation of

the points \mathbf{t}_j in equation (5). Assuming that $\mathbf{f}(\mathbf{t})$ is radially symmetric so that $\mathbf{f}(\mathbf{t}) = g(t)\mathbf{t}/t$ for $t = |\mathbf{t}|$ and some function $g(t) \geq 0$ with $g(t) \downarrow 0$ for sufficiently large $t \rightarrow \infty$, the sum for equation (5) is well defined and finite for all \mathbf{t} when, for sufficiently large a ,

$$\int_a^\infty t^{n-1} g(t) dt < \infty \quad (6)$$

Sums analogous to equations (1) and (2), that is,

$$\mathbf{Y}_R(\mathbf{t}) = \sum_{|\mathbf{t}_j| \leq R} \mathbf{f}(\mathbf{t}_j - \mathbf{t}) \quad (7)$$

have probability distributions with a limit for $\mathbf{t} = 0$ when

$$\int_a^\infty t^{n-1} g^2(t) dt < \infty \quad (8)$$

and there is a limit for all \mathbf{t} when, additionally,

$$\int_R^{R+a} t^{n-1} g(t) dt \quad (9)$$

has a finite limit as $R \rightarrow \infty$. These limiting distributions coincide for all \mathbf{t} when the limit of the integral (9) is zero.

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Giorgi System

THE letter by Stopes-Roe¹ on "Essential Features of the Giorgi System of Electromagnetism and a Basic Error by Sommerfeld" reveals that a bewildering number of formulations of the equations of electromagnetism are being advocated. Some related problems are discussed by Rosser².

In SI units the constant $\mu_0 = 4\pi \times 10^{-7}$ is indispensable in many formulae, if the dimensions are to balance and the correct numerical values are to be obtained. The difficulty arises from attributing to this constant the physical properties of permeability. As shown by Stopes-Roe, different definitions of magnetic moment lead to different versions of magnetic formulae; these definitions with their associated formulae are self-consistent and all equally correct in free space. If, however, μ_0 in the free space formulae is replaced by $\mu_0\mu_r$ in a medium of relative permeability, μ_r , the different systems predict different results. In particular the Kennelly formulation predicts that the field H due to a magnetic dipole is inversely proportional to μ_r , while the Sommerfeld formulation predicts that it is independent of μ_r .

I have so far failed to find references to any experimental evidence that would distinguish between these predictions. Because the experiments would have to use a fluid with μ_r very close to unity the experiments would be difficult, although probably not impossible.

Many older textbooks, such as Starling³, deduce from a dubious analogy with electrostatics that H is inversely proportional to μ_r . The electrostatic proof rests on the Gauss theorem in the form that the integral of the outward normal displacement D_n over a closed surface

$$\int_s D_n ds = 4\pi q \quad (1)$$

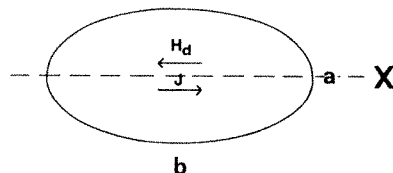


Fig. 1. Polarization and demagnetizing field in ellipsoid.

where q is the charge enclosed in e.s.u. This proof is inapplicable to magnetism because the equivalent formula is

$$\int_s B_n ds = 0 \quad (2)$$

Page and Adams⁴ deduce that the field outside a long thin magnet with approximately point poles is inversely proportional to μ_r , but from the nature of the approximations it is unlikely that this result holds for other shapes of magnet.

An illuminating example is provided by an ellipsoid with uniform polarization J in the X direction, as shown in Fig. 1. There is then a uniform demagnetizing field H_d inside the ellipsoid and using the Kennelly convention

$$B = J - \mu_0 H_d \quad (3)$$

The field H at any external point and the demagnetizing field H_d inside the ellipsoid can be calculated by integrating contributions from dipoles in small elements of volume throughout the ellipsoid, although sometimes the mathematics is simplified by using an equivalent distribution of poles on elements of surface of the ellipsoid. Whether the field is being calculated inside or outside the ellipsoid, the correct result is given by the free space formula independently of the relative permeability of the ellipsoid.

Now the Kennelly formulation requires that the field due to a point pole or dipole is inversely proportional to μ_r , when the ellipsoid is immersed in a medium. As a constant, $1/\mu_r$ can be taken outside the integral and therefore applies to the whole ellipsoid. Thus at any point outside the ellipsoid H is reduced by $1/\mu_r$ and $B = \mu_0 \mu_r H$ is unchanged. In particular this is true for an axial point a and an equatorial point b . Now at an equatorial point b , H is tangential to the surface of the ellipsoid and is therefore continuous across the boundary. So H_d is also reduced by the factor $1/\mu_r$. Inside the ellipsoid H_d is uniform, so H_d is also reduced by the factor $1/\mu_r$ at the axial point a just inside the ellipsoid. From equation (3), therefore, if J is unchanged, as is certainly possible in a hard magnetic material, B is increased by $H_d(1 - 1/\mu_r)$. Because B is normal to the surface of the ellipsoid at a and is continuous across the boundary, B is also increased outside the ellipsoid at a . Hence using the Kennelly formulation and the continuity relations, two contradictory results have been deduced. Outside the ellipsoid at an axial point a : (i) B is unchanged by a medium; (ii) B is increased by a medium. A similar reduction *ad absurdum* can be applied to the Sommerfeld convention.

The conclusion seems to be that we should not expect to find that the B and H produced by a magnet in a medium are simple functions of μ_r . They are more probably complicated functions of μ_r and geometrical factors depending on the shape of the magnet.

When a magnet is placed in a medium new dipoles are induced in the medium and those inside the magnet may or may not be altered. In general B and H can be calculated by taking account of all the macroscopic currents and magnetic dipoles that are near enough to be effective, using only the free space formulae.

This process would often be complicated, but the use of a formula involving μ_r or $1/\mu_r$ only as a simple coefficient is justified only when it leads to the same result. For the purpose of calculating B , H or magnetic forces (although not, of course, for some phenomena such as gyromagnetism), it does not matter whether magnetic dipoles are

spinning electrons or orbiting electrons. It is entirely a matter of mathematical convenience whether we treat them as dipoles or small current loops.

We may not, however, replace a magnet by a single solenoid, when we are considering conditions inside the magnet. These depend on the magnet consisting of many interacting dipoles or current loops. This interaction is lost if we postulate a single solenoid to represent the magnet.

In conclusion, an explanation of the physical difference between B and H in a magnetic material may be of interest. The flux density B is measured by a coil encompassing the material and in general is due to three causes: (i) external causes, such as a current in a solenoid surrounding the material. (ii) The self-demagnetizing field originating from discontinuities at the boundaries of the material. (iii) The local polarization J .

The field H is that which influences J by altering the orientation of the individual dipoles or current loops. An individual dipole or current loop can exert no torque upon itself, so that J does not contribute to H and

$$H = (B - J)/\mu_0 \quad (4)$$

I have constructed a model (yet to be published) consisting of a cubic array of small parallel magnets representing the atomic dipoles in a material. If one of these magnets is free to rotate, it turns so that it points in the opposite direction to the flux produced by the array as a whole, that is in the direction of H not B .

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Scattering of Gravitational Radiation by a Schwarzschild Black-hole

THE discovery of pulsars and the general conviction that they are neutron stars resulting from gravitational collapse have strengthened the belief in the possible presence of Schwarzschild black-holes—or Schwarzschild horizons—in nature, the latter being the ultimate stage in the progressive spherical collapse of a massive star. The stability of these objects, which has been discussed in a recent report¹, ensures their continued existence after formation. Inasmuch as the infinite redshift associated with it and its behaviour as a one-way membrane make the

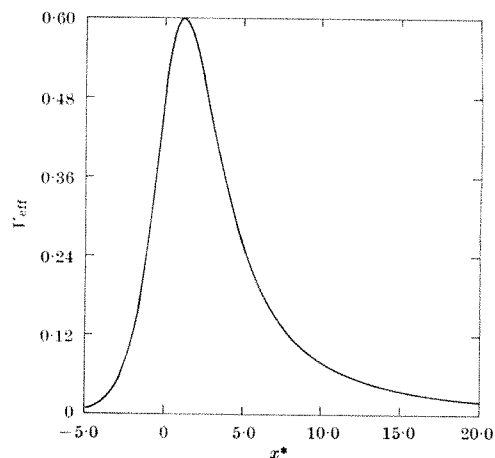


Fig. 1. The effective potential V_{eff} for the odd-parity gravitational waves of the lowest mode $l=2$ plotted against x^* .

Schwarzschild horizon at once elusive and intriguing, it is important to explore theoretically all possible modes in which the presence of such a black-hole manifests itself. In what follows, we present a partial summary of some results obtained from an investigation of the scattering of gravitational waves by a Schwarzschild horizon.

To begin with, a spherically symmetric mass distribution is assumed to have collapsed into its Schwarzschild surface in the infinitely remote past. The scattering of gravitational waves by this configuration can be examined employing the perturbation techniques developed by Regge and Wheeler². Retaining the notation of the authors and concentrating on the odd-parity waves, the perturbed Schwarzschild exterior line element in the Regge-Wheeler canonical gauge can be written as

$$ds^2 = -(1 - 2m/r)dt^2 + (1 - 2m/r)^{-1}dr^2 + r^2(d\theta^2 + \sin^2\theta d\varphi^2) \\ + (h_0(r) dt d\varphi + h_1(r) dr d\varphi) \exp(-i\omega t) \sin\theta \frac{dP}{d\theta} l(\cos\theta)$$

where ω is the frequency of the gravitational waves. The Einstein empty-space field equations computed to first order in the perturbations³ yield the following differential equations for the radial functions h_0 and h_1

$$\frac{d^2 Q}{dx^{*2}} + (k^2 - V_{\text{eff}})Q = 0 \text{ with } V_{\text{eff}} = \\ (1 - 1/x) (l(l+1)/x^2 - 3/x^3)$$

and

$$h_1 = \frac{i}{k} \frac{d}{dx^*} (xQ)$$

where we have defined

$$x = r/2m, \quad x^* = x + \ln(x-1), \quad k = 2m\omega, \quad \text{and} \quad Q = (1 - 1/x)h_1/x$$

The exterior from $r = 2m$ to ∞ corresponds to the range of x^* from $-\infty$ to $+\infty$. The motion of the gravitational waves in this space is governed by the effective potential V_{eff} produced by the collapsed mass. The effective potential for the lowest possible mode $l=2$ is plotted against x^* in Fig. 1, and the general behaviour of the potential for any higher value of l is the same, it is positive and goes to zero asymptotically as x^* approaches $\pm\infty$ and attains a maximum in between. From the Schrödinger form of the wave equation for Q and from the shape of the potential, it is evident that the scattering problem here is formally the same as that encountered in quantum mechanics for a one-dimensional potential barrier. A wave coming from spatial infinity is partially reflected by the effective potential, so that at large values of x we have both incoming and outgoing waves. On the other hand, as the Schwarzschild horizon acts as a sink for the radiation, there will be only waves entering the $r = 2m$ surface. Consequently the suitable asymptotic boundary conditions are $Q_\infty = A(k)e^{-ikx^*} + B(k)e^{ikx^*}$ and $Q_\infty = C(k)e^{-ikx^*}$ for x^* approaching $\pm\infty$ respectively. In analogy with the quantum mechanical problem we can define the reflexion and transmission coefficients $R = |B/A|^2$ and $T = |C/A|^2$. A fraction R of the incident wave escapes to spatial infinity and is accessible to a distant observer, whereas a fraction T of the radiation is absorbed by the black-hole and thereby lost in the process. An analytical integration of the equation for Q leading to the computation of R and T has been impossible in practice and recourse had to be taken to numerical integration. This has been carried out—as have further computations to be discussed later—for the lowest mode $l=2$ using a computer. Fig. 2 shows the plot of R against k^2 . In the limit of zero frequency the reflexion coefficient approaches the limit 1 independent of the scattering mass, and so in this limit no information about the latter is forthcoming. Nevertheless, the rate at which the reflexion amplitude B/A , and so R , decreases as a function of the frequency should be perceptible when a sufficient range of frequencies is included and, because this rate clearly depends on the scattering mass, the presence of the latter is “coded” into the outgoing radiation. This leads us at once to the

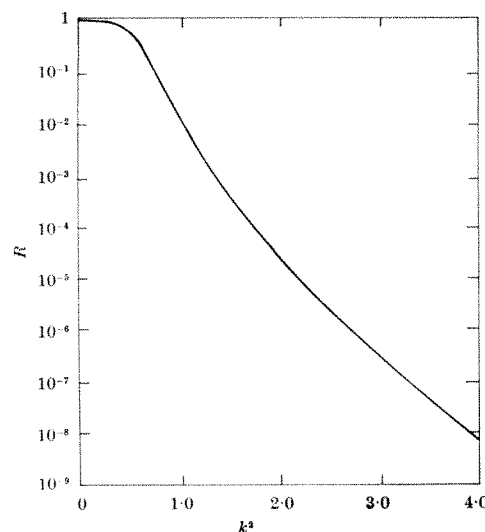


Fig. 2. The reflexion coefficient R as a function of k^2 , the square of the frequency, for odd-parity mode $l=2$.

physically more interesting phenomenon of the scattering of wave packets. By linear superposition we obtain an incoming wave packet at infinity with the spatial profile

$$\psi_{\text{in}}(x) = \int_{-\infty}^{+\infty} f(k)e^{-ikx} dk$$

and the corresponding outgoing or reflected packet

$$\psi_{\text{out}}(x) = \int_{-\infty}^{+\infty} (B/A) f(k)e^{ikx} dk$$

The latter travels out without spreading and is received by the distant observer. We choose the Gaussian function

$$f(k) = \frac{1}{2\sqrt{\pi}a} e^{-k^2/4a}$$

in order to obtain a simple model for an incoming wave packet, that is, $\psi_{\text{in}} = e^{-ax^2}$. As the parameter a , which measures the width of the wave packet, is varied, some interesting features emerge. For low values of a ($a \lesssim 0.01$), that is, for very broad incoming packets or equivalently for $f(k)$ sharply peaked at zero frequency, the reflected packet is practically unaffected. But, as the parameter a is gradually increased, ψ_{out} develops distinct maxima and minima that increase in number progressively, while their relative spacing undergoes a continuous change. As the parameter a , however, approaches approximately the value 1, that is, for a width of about the Schwarzschild radius, the process reaches a limit. Beyond this value of a , as the packet is made thinner, the outgoing packet will cease to develop new peaks and the relative spacing of these peaks will remain unaltered. In other words, any higher frequencies added to the original packet will have negligible effect on the scattered packet owing to their almost total absorption by the black-hole. As long as the incoming packet is spatially sharp enough, the reflected packet will manifestly carry information about the scattering mass. Fig. 3 shows an example of the “saturated” pattern corresponding to $a=1$. The spacing between consecutive peaks and, consequently, the lag in their arrival times are measures of the scattering mass, as the spacing in the actual radial distance is given by $\Delta r = 2m \Delta x$.

The total energy carried by a wave packet $\psi(x)$ at spatial infinity can be computed by adapting a method used by Edelman⁴. The result of this computation is that, for any mode l , the energy of the wave packet is given by

$$E = (c^5/32\pi G) (l-1)l(l+1) \int (\psi(x))^2 dx$$

where the integration is carried over the spatial extent of the wave packet. So the fraction of incident radiation scattered by the black-hole and reaching spatial infinity

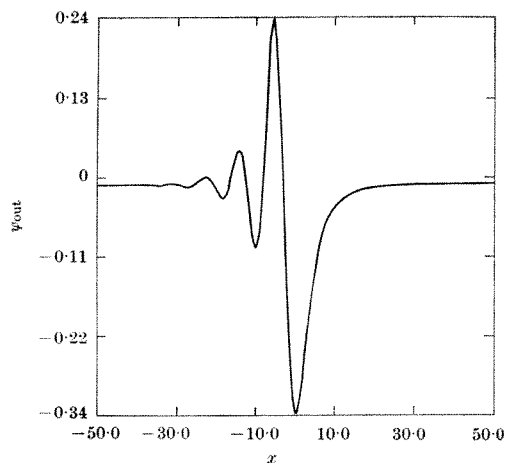


Fig. 3. The outgoing wave packet $\psi_{out}(x)$ at spatial infinity corresponding to the incident Gaussian wave packet $\psi_{in}(x) = e^{-ax^2}$ with $a=1$.

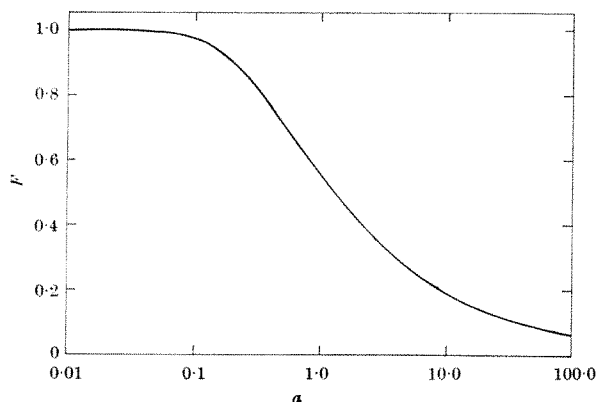


Fig. 4. The fraction F of the incident energy carried by the scattered outgoing wave packet at spatial infinity plotted as a function of the parameter a .

is the ratio of the energies carried by the incoming and the outgoing wave packets and is readily computed as

$$F = \frac{\int (\psi_{out})^2 dx}{\int (\psi_{in})^2 dx} = \left(\frac{2a}{\pi}\right)^{1/2} \int (\psi_{out})^2 dx$$

In Fig. 4 the fraction F is plotted as a function of the width-parameter a . For an incident wave packet, the width of which is about a Schwarzschild radius ($a \approx 1$) approximately half the total energy is scattered and the rest absorbed by the black-hole.

We have confined ourselves so far to some results concerning the scattering of odd-parity gravitational waves of angular momentum $l=2$ by a Schwarzschild black-hole. The mathematical and numerical details omitted here, as well as the scattering of higher l modes, even-parity waves, scalar gravitational waves and finally electromagnetic waves, will be discussed elsewhere in a separate and more detailed paper.

I thank Professor C. W. Misner for many helpful discussions, Dr A. Lapidus and Mr Gary Russel for their help in carrying out numerical computations, and the authorities of the NASA Institute for Space Studies, New York, for allowing me to use their computer. The research was supported by funds from the US National Science Foundation.

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Phase Change in the Upper Mantle above 350 km

BULLEN^{1,2} has used the Adams-Williamson relation in region B (upper 400 km in the mantle) to relate the rate of change of density in the Earth's interior to the rate of change of seismic velocity. The Adams-Williamson relation implicitly assumed that phase changes are absent. Recent interpretations of the velocity gradient in the mantle³ have re-emphasized the importance of phase changes in region C (between 400 and 900 km). Region B is often treated as a homogeneous layer, but I present here some evidence to show that phase change may also occur in this region.

A number of empirical equations which relate densities of common rocks and minerals with velocities of compressional waves⁴⁻⁶ show that velocity is linearly proportional to density

$$V_p = A + B\rho \quad (1)$$

where B has a value of about 3 (km/s)/(g/cm³). This relation has been applied to the calculation of density in various regions of the Earth from known seismic velocities⁷⁻⁹. For common rocks and minerals, bulk

sound velocity, C , which is defined as $(V_p^2 - \frac{4}{3}V_s^2)^{1/2}$, was also found to be linearly proportional to density. This relation may be expressed as

$$C = a + b\rho \quad (2)$$

where, for rocks and minerals with mean atomic weight about 21, b has a value of 2.36 (km/s)/(g/cm³) (ref. 10). This empirical relation was shown to follow closely the C versus ρ relation for a single material under large compression^{11,12}. Anderson¹³ gave a seismic equation of state relating the seismic parameter φ (which is identical to C^2) and ρ

$$\varphi/\varphi_0 = (\rho/\rho_0)^n \quad (3)$$

Fitting this equation to data for thirty-one selected rocks and minerals, the value for n is found to be about 3.

With increasing depth, both temperature and pressure rise. The implicit assumption in the application of any one of the empirical density velocity relations to the prediction of density in the upper mantle is that the effect of an increase of temperature and pressure with depth might change density and velocity in approximately the same ratio as that represented by the empirical relations. I have found¹⁴ that, in regions of high temperature gradient such as the upper mantle, this assumption is violated for the relation between V_p and ρ but is obeyed for equations 2 and 3. These relations are used to estimate the density differences at various depths in the upper mantle, corresponding to a given distribution of seismic velocities.

As noted by Anderson¹³, the seismic equation of state tends to predict smaller change of density than does Birch's equation of state at a given change of φ . In Fig. 1, ρ/ρ_0 is plotted against φ/φ_0 for the following equations: equation 6, with $b=2.36$ (km/s)/(g/cm³) and $C_0/\rho_0=1.8$ (km/s)/(g/cm³) as for most rocks and minerals (a is adjusted such that $C=C_0$ when $\rho=\rho_0$), equation 3 ($n=3$), and finally Birch's equation¹⁵

$$\varphi/\varphi_0 = \frac{1}{2}(\rho/\rho_0)^{\frac{2}{3}}[7(\rho/\rho_0)^{\frac{1}{3}} - 5] \quad (4)$$

Fig. 1 shows that in the range of interest for this study, the values for ρ/ρ_0 corresponding to equation 4 always fall between those corresponding to equations 2 and 3, with differences less than 3 per cent. Equation 2 always yields greater values for ρ/ρ_0 than does equation 3, with differences less than 5 per cent. The use of both equation 2 and equation 3, for the prediction of density in the upper mantle, probably would bring out an adequate range of uncertainty in the predicted densities due to the uncertainty in the density-velocity relations.

Table 1. VALUE OF C AND φ USED IN THIS STUDY

Depth (km)	Gutenberg		Johnson, V_p , and Ibrahim and Nuttli, V_s	
	C (km/s)	φ (km/s) ²	C (km/s)	φ (km/s)
150	6.03	36.4	5.94	35.3
350	6.82	46.5	6.90	47.6

The distribution of seismic velocities is critical for our study. Recent studies on body wave and surface wave data have given strong evidence for the existence of an extensive low velocity layer not far below the Mohorovicic discontinuity, thus lending support to Gutenberg's, rather than Jeffreys's, velocity distribution in the upper mantle. I chose two sets of C distributions (Table 1): one is from a combination of Gutenberg's¹⁶ V_p and V_s distribution; the other is from a combination of Johnson's¹⁷ CIT 204 for V_p and Ibrahim and Nuttli's¹⁸ V_s . The latter set is characteristic for the upper mantle beneath the western United States. The differences between these two sets of C distribution should give some indication of the uncertainty in the seismic models. Between 150 and 350 km, the differences in C , which correspond to these two sets of C distribution, are, respectively, 0.8 and 1.0 km/s; the ratios between the two values of φ at these two depths are, respectively, 0.78 and 0.74. Corresponding to equation 2 with $b = 2.36$ (km/s)/(g/cm³), the difference in density between 150 and 350 km is $\Delta\rho = 0.34$ and 0.41 g/cm³, respectively. Density at 150 km is about 3.3 to 3.5 g/cm³. Thus

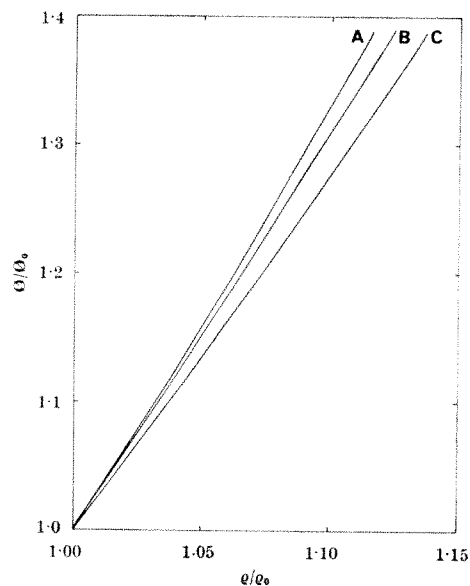
$$\frac{\Delta\rho}{\rho_0} = 10 \text{ to } 12 \text{ per cent}$$

where the subscript 0 refers to the density at 150 km. According to the seismic equation of state (equation 3, $n = 3$), we obtain, respectively,

$$\frac{\Delta\rho}{\rho_0} = 8 \text{ to } 10 \text{ per cent}$$

Thus if the assumption of nearly uniform iron content in the upper mantle is correct, the increase of density between 150 and 350 is somewhere between 8 and 12 per cent.

If there are significant changes of mean atomic weight with depth in the upper mantle, the calculation breaks down. Greater density for a given velocity usually corresponds to greater mean atomic weight, and vice versa. Thus if there is an increase of mean atomic weight with depth in the upper mantle, the actual density change would be greater than those calculated above. On the other hand, if there is a decrease of mean atomic weight with depth, then the actual density change would be smaller than those predicted by the empirical equations. Present evidence for significant change of mean atomic weight with depth in the upper mantle is, however, almost totally absent. Although evidence on the uniformity of mean atomic weight in the upper mantle is equally meagre, the assumption of uniform mean atomic weight is the least complicated. Furthermore, Earth models with an increase of density of this magnitude between 150 and 350 km have been found which satisfy all relevant geophysical data including the periods of free oscillations, the phase and group velocities of surface-

Fig. 1. ρ/ρ_0 plotted against v/v_0 for equations 2, 3 and 4.

waves, and the total mass and moment of inertia of the Earth¹⁴.

The composition of the upper 400 km of the mantle is usually considered to resemble that of some varieties of peridotite in which olivine is the predominant constituent. Thus the effect of gravitational self-compression between 150 and 350 km may be estimated, to a good approximation, from that for a homogeneous layer of olivine. The isothermal bulk modulus for olivine at 1 atmosphere and room temperature is 1,270 kb (ref. 19). Temperature at the depth of 150 km is about 1,500° C (ref. 20). Using $(\delta K_T/\delta T)_P = -0.208$ kb/deg (ref. 19), the value of K_T at this temperature becomes 950 kb. The corresponding value at a higher pressure may be estimated. The experimental value of $\delta K_T/\delta P$ for olivine is 5.1 at room temperature¹⁹. The change of this parameter with temperature, however, is unknown. I used the values $(\delta K_T/\delta P)_T = 4$ at $T \approx 1,500^\circ \text{C}$. Thus the value of K_T for olivine at 150 km, where the pressure is about 50 kb, is 1,150 kb. This value of K_T may be used to estimate the change of density from 150 to 350 km from isothermal compression alone; the result will be corrected later for the effect due to the difference in temperature between these depths. Birch's equation of state¹⁵ is used to calculate the change of density caused by isothermal compression

$$\frac{2}{3} \frac{\Delta P}{K_T} = \left(\frac{\rho}{\rho_0} \right)^{\frac{2}{3}} - \left(\frac{\rho}{\rho_0} \right)^{\frac{1}{3}}$$

The pressure difference between 150 and 350 km is about 70 kb (refs. 10, 21, 22). The corresponding value for ρ/ρ_0 is 1.056, where the subscript 0 refers to the density at 150 km. Thus the maximum change of density due to self-compression in this depth range is 5.6 per cent. Assuming a temperature difference of 300 degrees¹⁵ and using a coefficient of 2×10^{-5} per degree for thermal expansion, it follows that $\Delta\rho/\rho_0 = 5$ per cent, where $\Delta\rho$ is the change of density from 150 to 350 km from self-compression.

Thus about one third to two thirds of the total increase of density between 150 and 350 km (8 to 12 per cent) cannot be accounted for by the self-gravitational compression and may be associated with phase transitions in mineral assemblages occurring in this range of depth. Considerable discussion has been given to the breakdown of aluminous pyroxene to pyrope-garnet plus low-alumina pyroxene^{23, 24}. Whether this suggestion is sufficient to account for the density change due to phase

transition as indicated in this study is not discussed here.

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Thermoluminescence Dating applied to Volcanic Lava

THE thermoluminescence dating technique applied to volcanic lava has been reported by Aitken *et al.*¹ In their preliminary experiments, grain sizes of between 40 and 80 μ m in diameter were selected by Franz-type magnetic separators, and a spurious glow was observed even from some young lavas. This spurious glow was attributed to the impurities which are difficult to separate from the luminescent grains with magnetic separators.

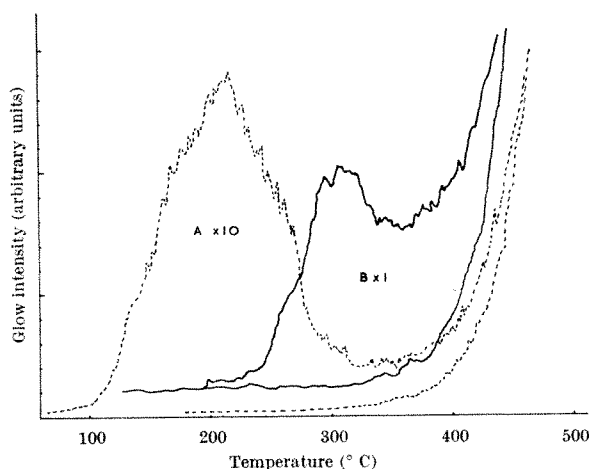


Fig. 1. A, Artificial TL glow (250 rads beta, ⁹⁰Sr); B, natural TL glow of feldspar from the Pompeii ash.

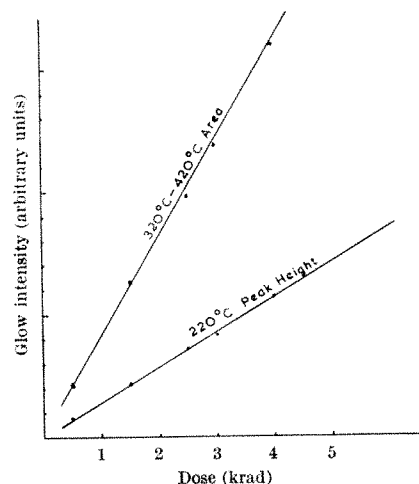


Fig. 2. Susceptibility of the feldspar to radiation dose (⁹⁰Sr source).

Our new approach to this technique of volcanic rock dating is to select crystalline grains from a rock for measurement of the thermoluminescence. Three kinds of crystals are commonly found in lava; they are light green, dark green or white in colour. The volcanic ash we examined first is the one believed to have destroyed Pompeii. The needle shaped, transparent feldspar from this ash was identified as orthoclase by X-ray diffraction and optical methods. The other coloured crystals were found to be insensitive to the thermoluminescence effect.

Ten to twenty crystals of 0.5 to 1 mm in diameter were selected for thermoluminescence measurement. The crystals were ground with 600 grade carborundum and water so that they had a flat bottom in order to make good contact with the tantalum heating strip. This arrangement also provided a repeatable geometry for the thermoluminescence measurement. The induced thermoluminescence by the natural radioactive isotopes or by artificial irradiation was measured conventionally by a photomultiplier tube and the heating rate used was 20° C/s in an atmosphere of nitrogen. The natural glow and artificial glow of a sample are shown in Fig. 1. The scale of the glow intensity for curve A should be multiplied by 10 when it is read. The 220° C peak induced by artificial irradiation was not found in the glow curve because it was drained by the geological temperature during burial. For dating purposes, the total glow in the temperature region between 320° C and 420° C was chosen, using the "ordinate ratio test" technique². The ratio of the natural glow to the artificial glow at the same temperature was plotted against the heating temperature and a plateau appeared above 320° C. In this way, the thermal bleaching during burial is assumed not to affect the trapped carriers. No allowance, however, has been given for the possibility of the decay of trapped carriers by the tunnelling effect, which is believed to be negligible. The susceptibility of the sample to radiation was calibrated using a ⁹⁰Sr beta source and is shown in Fig. 2. The age determination of the sample is based on the assumption that the beta and gamma particles have the same effectiveness in inducing thermoluminescence. Also, because grains are chosen so that their diameters are much bigger than the range of the alpha particles from the natural content of uranium and thorium, the annual dose contributed by the alpha particles is considered to be insignificant³. The age of this sample was found to be of the right order using these assumptions. The most significant result is that the other lavas, which were

Table 1

Sample	Annual dose (rad/year) (U + Th + K + cosmic ray)	Archaeological age (year)	TL age (year)
Ash	1.1596	1821	1797 ± 160
Grey lava	2.444	64 ?	0
Brown lava	1.886	64 ?	0

collected on the surface of the upper slope of Vesuvius, and are believed to be young (the latest major eruption was in 1906), did not show any spurious glow, although the magnetically separated portion of these samples did give a spurious glow.

I thank Dr G. L. Hendry of the Department of Geology of the University of Birmingham for the X-ray diffraction and the potassium content analysis, Dr J. I. Langford of the Department of Physics for optical identification of the mineral and Miss J. Thompson of the University of Oxford for the uranium and thorium content measurements. A full programme is under way for examining more samples, and I would like to appeal for the donation of volcanic ashes of known ages in order to establish the technique.

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Long-lived γ -Ray Emitting Nuclide Silver-108m found in Pacific Marine Organisms and used for Dating

DRIED tissues of several marine organisms collected in 1964 from widely separated regions of the North Pacific have turned out to contain ^{108m}Ag , a γ -ray emitting man-made nuclide with a half-life reported¹ to be greater than 100 yr. This seems to be the first observation of ^{108m}Ag in any organism as the apparent consequence of global fallout. This nuclide promises to be useful for large scale labelling and dating. We have used it to follow the progress of a specific oceanic water mass.

The short-lived (253 days) ^{110m}Ag was determined in 1964 (ref. 2); on the other hand, a recently installed two-dimensional γ -ray spectrometer of a type developed by the Hanford group³ was used for measuring ^{108m}Ag . Table 1 lists the activities of ^{108m}Ag found in samples of squid, mussel, lobster, and tuna fish collected in 1964–65 at widely separated oceanic and coastal locations. The concentrations of ^{110m}Ag measured in 1965–66 are also listed, together with the individual activity ratios. All activities were extrapolated arbitrarily to conditions on August 7, 1964, when the first oceanic squid sample² to reveal ^{110m}Ag was collected. The last column of Table 1 contains the dates of apparent nuclear origin implied by the activity ratios according to the assumptions indicated. Table 2 describes the specimens with regard to place of collection, species and sample size in terms of the wet weight of the organs counted.

Table 1. CONCENTRATIONS OF SILVER-110m AND SILVER-108m IN "LIVER" TISSUE

Fig. 1 position	Organism yielding "liver" tissue sample	Silver activities* of sample extrapolated to August 7, 1964		Ratio of sample activities on August 7, 1964 (Ag-110m/Ag-108m)	Date of original activation calculated from August 7, 1964, activity ratio†
		Ag-110m (pCi/sample)	Ag-108m (pCi/sample)		
A	Squid from NE Pacific	360 ± 15	15.5 ± 0.9	23.2 ± 1.7	Aug. 25, 1962 (± 30 days)
B	Squid from NE Pacific	87 ± 6	4.9 ± 0.5	18 ± 2	May 24, 1962 (± 40 days)
C	Yellowfin tuna from Cape San Lucas area	20 ± 4	1.07 ± 0.17	19 ± 5	June 13, 1962 (± 100 days)
D	Lobsters from Guadalupe Island ‡	150 ± 8	8.1 ± 0.5	18.5 ± 1.5	June 3, 1962 (± 30 days)
E	Albacore caught off San Diego, rpl. 1	26 ± 3	2.9 ± 0.7	9 ± 2	Sept. 12, 1961 (± 80 days)
F	Albacore caught off San Diego, rpl. 2	26 ± 3	2.9 ± 0.6	9 ± 2	Sept. 12, 1961 (± 80 days)
				Mean	Mean ± 55 days
G	Byssal mussels from W. side of Okinawa§	51 ± 4	4.2 ± 0.4	12.1 ± 1.5	Dec. 29, 1961 (± 50 days)

* One sigma standard deviation of counting including instrument background consideration.
† Based on assumption of single activation of natural silver to initial activity ratio from reactor (Ag-110m/Ag-108m = 162), followed by decay with half-lives of 252.5 ± 1.5 days¹ for Ag-110m and 127 ± 7 years¹ for Ag-108m.

‡ Hepatopancreas.

§ Whole soft tissue.

Table 2. DESCRIPTION OF SPECIMENS

Fig. 1	Position	Collected	Species	Individuals	Liver weight*
A	34° N 135° W	7 Aug. 64	<i>Stenoteuthis bartrami</i> (squid)	1	103
B	39° N 138° W	25 Feb. 65	<i>Stenoteuthis bartrami</i> (squid)	1	30
C	23° N 110° W	15 June 64	<i>Neothunnus macropterus</i> (tuna)	4	415
D	29° N 118° W	2 Mar. 65	<i>Panulirus interruptus</i> (lobster)	6	147
E	33° N 117° W	26 July 64	<i>Thunnus alalunga</i> (albacore)	3	325
F	33° N 117° W	26 July 64	<i>Thunnus alalunga</i> (albacore)	3	318
G	27° N 128° E	2 Sept. 64	<i>Volvella agripeta</i> † (mussel)	40	315

* Wet weight (g).

† Whole soft tissues.

Table 1 lists the usual estimated (one sigma) standard counting errors. The uncertainties in the date values in the last column were computed from the errors of the ^{108m}Ag and ^{110m}Ag activities. Uncertainties in half-life should affect the earliest date by no more than 15 days and other dates by even less. An initial $^{110m}\text{Ag}/^{108m}\text{Ag}$ activity ratio of 162 was assumed in the dating computations, this being the value reported immediately after activation of natural silver in a reactor experiment⁴. It is difficult to speculate on the similarities and dissimilarities between the conditions existing in a reactor and during the explosion of a nuclear weapon, but, taking into account the natural abundances of ^{107}Ag and ^{109}Ag and the decay rates of ^{110m}Ag and ^{108m}Ag , an initial activity ratio of 162 implies very nearly equal activation cross-sections for the production of ^{110m}Ag and ^{108m}Ag . In view of this and the reported⁵ similarity between the nuclear structures of the two sets of silver states, it seems reasonable to use 162 in the case of nuclear weapons.

^{108m}Ag was calibrated by standardization of the two dimensional spectrometer with a source of pure ^{108m}Ag generously supplied by the Los Alamos Scientific Laboratory. This source was first calibrated at Scripps by comparison with a standardized point source of ^{137}Cs (using the ^{108m}Ag decay scheme shown by Lederer, Hollander and Perlman⁶, with an EC/IT branching ratio of 91.5/8.5 (ref. 4)). In 1964, ^{110m}Ag had been calibrated by reference to the same source of ^{137}Cs . Our experience⁶ suggests that the absolute accuracy of calibrations of standards of this sort usually are not better than ± 5 per cent.

The salient feature of ^{108m}Ag is that it is an artificial γ -ray emitter with an exceptionally long life, longer than any other man-made γ -ray emitter reported anywhere in the biosphere. The slow decay of ^{108m}Ag suggests that it would be useful, with ^{110m}Ag , for very large scale labelling and experiments. Both these nuclides seem to be activated together when ordinary silver is exposed in reactors and in the detonation of certain weapons.

An idea of the use of $^{110m}\text{Ag}/^{108m}\text{Ag}$ dating can be gained by considering how the animals listed in Table 1 could have come into contact with the observed silver nuclides. This will require reference, first, to reported dates when specific large weapons were tested; second, to the reported character of the global fallouts that followed; third, to the motion and stratification of upper layers in the Pacific;

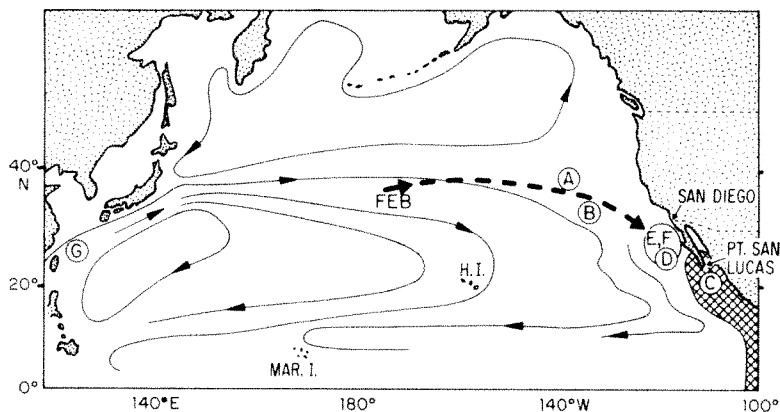


Fig. 1. Locations where organisms contained long lived silver radionuclide were collected. Arrows show generalized major oceanic circulations; circles show collection locations; cross-hatching shows yellowfin tuna fishery regions; - - - , suspected spring migration of albacore caught off California (regions E, F) June-July albacore fishery.

and fourth, to the probable environment and characteristic behaviours of the several organisms.

The testing of very large nuclear weapons was renewed abruptly on October 23, 1961; other tests were made in 1962, especially in the autumn. The observed silver nuclide ratios in Table 1 indicate the absence of large amounts originating in 1958 and earlier. Thus the observed silver nuclides most likely originated within the 1961 and 1962 testing periods.

After the 1961-62 weapons tests, there were intense global fallouts during the spring of 1962, 1963 and 1964, especially intense at latitude 40°-50° N (ref. 7). Presumably the observed ^{108}mAg and ^{110}mAg could have entered the sea any time after October 23, 1961, but the largest fraction of silver of 1961 origin should have entered in the spring of 1962. Lesser amounts that had been stored in the stratospheric reservoir should then have followed late in 1962, and also in 1963 and 1964. But, after the summer of 1962, and especially after October 1963, all the 1961 fallout must have been mixed with some that originated in 1962. This mixture would be expected to have entered the ocean most rapidly in the spring of 1963, yet some with slightly different silver nuclide mixtures should have fallen later, especially in the spring of 1964.

Finding mixtures of silver nuclide ratios in organisms corresponding closely to late origin therefore indicates that either very little radioactive silver was formed in 1961 compared with 1962, or the species with the late origins lived in regions where silver was dispersed or depleted rapidly, and they adjusted rapidly to changes in silver in their environment. The first alternative is unlikely because of findings in the case of the albacore, as will be shown later.

Table 1 shows that all but one of the species inspected had large fractions of 1962 fallout as indicated by the radio-silver ratios. One squid (A) contained a nuclide ratio that might have come from the autumn of 1962 when the final large tests of this post-moratorium series took place. This squid was feeding at the surface when captured. It seems therefore that its feeding region was being rapidly depleted of silver (and perhaps of other trace metals), and also that the biological half-life of the species was short—of the order of a few weeks or months.

Table 1 shows that another squid (B), some tropical tuna (C) and some lobsters (D) from a small island also contain silver nuclide ratios that suggest associations with water masses which were constantly depleted of silver, again suggesting rapid turnover of silver traces in the organisms or in their food. The lobsters of Guadalupe (D) usually live in shallow layers, and presumably, from the silver nuclide ratio found, they depend on food with similar environment. Yellowfin tuna are caught at the surface; the silver ratios they contain suggest that

their food comes from regions where there is a rapid turnover of silver. Another interesting factor in the environment of this population will be discussed later.

Byssal mussels (G) were collected from tide pools in a calcareous apron of Okinawa where rain containing relatively high concentrations of fallout struck the organisms directly during periods of low tide, and might also have accumulated and been retained in their fixed substrate and in other sessile organisms living nearby. In addition to recent fallout, therefore, early fallout rich in 1961 silver nuclides could well have remained available to the mussels until 1964 when they were collected. Their nuclide ratio of intermediate value suggests this possibility.

The two albacore individuals (E and F) had a remarkably different silver nuclide ratio that can best be understood with reference to surface and subsurface surveys of ^{137}Cs in the Pacific^{8,9}. It can be shown that the ratio $^{110}\text{mAg}/^{108}\text{mAg} = 9$ corresponds within error to activation on October 23, 1961, when the first large nuclear test after the moratorium took place. This strongly suggests that these albacore individuals had recently been in contact, directly or through their food, with "almost pure" 1961 fallout silver radionuclides. Because completely unmixed 1961 fallout could not have entered the ocean later than the summer of 1962 (and presumably the largest fraction entered still earlier during the intense 1962 spring fallout peak), these albacore had evidently encountered and been exposed to a body of water or food that had been quite isolated for at least 2 yr from sources of recent fallout.

In Fig. 1 a suspected¹⁰ route of annual migration of the albacore population of the North Pacific is sketched (as a dashed line) leading into the June-July fishing ground (E, F) where the two individuals of Table 1 were caught. Albacore fishing usually begins in this general area, and then moves northward along the coast during the summer. Finally, the albacore apparently return to the central North Pacific later in the autumn. The habits of the fish immediately before appearing off Southern California and Baja California are not well known. But the fish are caught on the surface thereafter.

A mechanism now can be suggested to explain how a trace element might be isolated and kept pure quite effectively for several years in thin subsurface layers while moving thousands of miles, and thus how the albacore could have encountered essentially pure 1961 radioactive silver. The North Pacific circulation brings northern surface waters, and also the shallow subsurface layers supporting most of the marine organisms, eastward and southward past California. Fig. 1 shows the generalized circulation^{11,12} and also the collecting locations of the present experiment. It has become evident from surveys^{8,9} of fallout ^{137}Cs that fallout distributions in the North Pacific depend, after a few years, on oceanic circulation as well as on the distribution of initial fallout inputs. Relatively high inputs of fallout entering the sea near 40° N apparently are transported eastward and southward to latitudes of 10°-20° N, and much is then carried westward in low latitudes. Surface and shallow subsurface ^{137}Cs distributions measured in 1964-68 suggest transport rates for this movement of 2-3 cm/s, consistent with hydrographic predictions^{11,12}.

Detailed sampling of ^{137}Cs at numerous depths above 300 m has revealed thin stratifications delineated by high concentrations of ^{137}Cs most commonly between about 70 and 150 m. In overlying waters ^{137}Cs frequently was much less concentrated. These high subsurface concentrations of ^{137}Cs frequently were associated with salinity minima which have long been attributed to high rainfall in northern

latitudes. By 1968, the most intense subsurface ^{137}Cs stratifications were found between 20° and 10° N, and as far west as the longitude of Hawaii. When this 1968 picture of isolated and moving lenses or strata of fallout lying at shallow depths (as suggested by hundreds of measurements of ^{137}Cs in the Pacific) is extrapolated back to the summer of 1964, it is apparent that the moving front of the water contaminated by early post-moratorium fallout near 40° N must have reached the latitudes of the June–July albacore fishery (E, F) south of San Diego about when the albacore of Table 1 were caught. That is, the oldest fallout of 1961 and spring 1962 origin would have entered the region, much of it isolated below the mixing forces in the surface layers. These albacore must have had a unique opportunity for contacting almost unmixed 1961 fallout in 1964.

The situation of the yellowfin tuna is somewhat different. This is a tropical fish ranging to the equator and scarcely reaching temperate latitudes (Fig. 1). Individuals reaching Point San Lucas by summer 1964 were just too early to meet the old fallout front borne by currents coming down from the north. The yellowfin tuna must have been exposed, however, to aerial fallout containing young 1962 silver activities.

No evidence of hazard to humans should be inferred from our finding of long-lived $^{108\text{m}}\text{Ag}$ in commercially collected tuna. The activities we measured were so small as to be difficult even to detect—very much smaller than activities that are natural to the tissues.

We thank Professor C. L. Hubbs for collecting lobster tissues, and Professor C. L. Osterberg for a squid specimen. Many people helped to collect the other samples. We also thank Drs M. E. Bunker, J. W. Starner and S. G. Schmelling for sources of $^{108\text{m}}\text{Ag}$. Dr G. Harbottle sent his unpublished report¹ concerning decay of $^{108\text{m}}\text{Ag}$ and also several other valuable references. This work was supported by the US Atomic Energy Commission and the US Office of Naval Research.

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Stable Isotope Study of the Palaeoenvironment of the Neolithic Site of Nea Nikomedeia, Greece

THE Neolithic mound of Nea Nikomedeia¹ lies some 25 km north-west of the mouth of the river Aliakmon, in the Macedonian Plain of Greece. Field evidence suggests that at the time of the Neolithic occupation the low land near the site, drained in the last century, may have been

occupied either by a lake or by an arm of the Gulf of Thermai².

Although shellfish remains are not abundant in the deposits, shells of the common cockle, *Cerastoderma edule* (Linne), are sufficiently abundant in some areas to indicate that they were eaten. Because the site is distant enough from the present coastline to have dissuaded prehistoric man from carrying back shellfish before eating them, it seemed likely that any information relating to the habitat of these cockles might shed light on the nature of the water body nearer the site. To this end, oxygen and carbon isotopic analyses of samples of a few of the shells have been carried out.

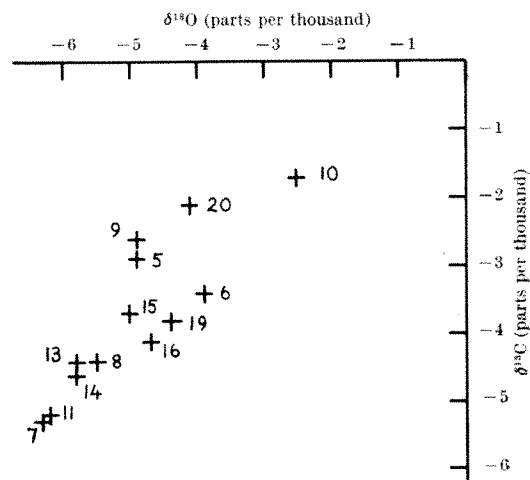


Fig. 1. Oxygen and carbon isotopic composition of the edges of cockle shells (*Cerastoderma edule*) from Nea Nikomedeia, expressed as a deviation in parts per thousand from the PDB standard.

The basis of this approach is a systematic survey by Keith *et al.*³, who showed that a knowledge of the carbon and oxygen isotopic composition is sufficient broadly to characterize the aquatic environment of a mollusc. In Fig. 1, the oxygen and carbon isotopic composition of samples taken from the edges of a number of shells from the site of Nea Nikomedeia are shown. It will be seen that the range of oxygen isotopic composition ($\delta^{18}\text{O}$) is from -1 to -5 parts per thousand, and that the range of carbon isotopic composition ($\delta^{13}\text{C}$) is from -3 to -7 parts per thousand, both relative to the PDB standard⁴. These values suggest an estuarine environment. (Precise calibration in terms of the PDB standard is difficult because none of the original material is now available. The definition of the standard is also generally regarded as implying a particular extraction procedure including reaction at 25°C , and differing in several respects from that adopted here. For the purposes of this work, the precise meaning of the zero point in Figs. 1 and 2 is unimportant.)

More detailed work on shells from an estuarine environment was carried out by Mook and Vogel⁵, who analysed shells collected at varying distances from the sea along two estuaries adjoining the North Sea. Their work confirmed that these molluscs deposited both carbon and oxygen in the carbonate of their shells in isotopic equilibrium with the bicarbonate ion in the water in which they grew. The oxygen isotopic composition of the water and hence of the bicarbonate varies with distance from the sea, as the relative proportion of sea water, $\delta^{18}\text{O} \approx 0$, and fresh water, $\delta^{18}\text{O} \approx -10$ parts per thousand, varies. At the same time the carbon isotopic composition changes, for the bicarbonate in sea water has $\delta^{13}\text{C} \approx +2$ parts per thousand, while in river water the bicarbonate has $\delta^{13}\text{C} \approx -12$ parts per thousand as a result of the incorporation of isotopically light carbon derived from the decomposition of plant matter.

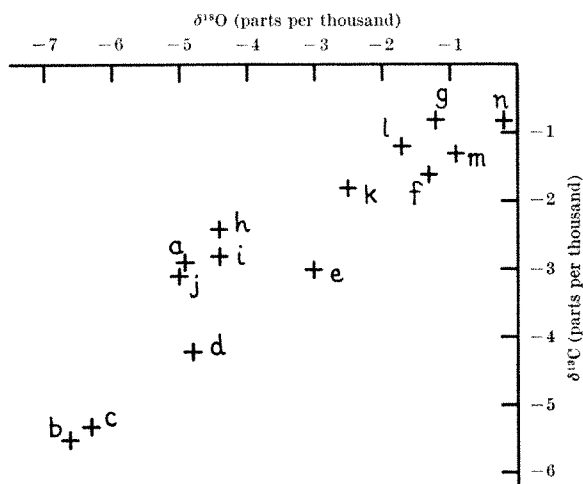


Fig. 2. Oxygen and carbon isotopic composition of sequential growth increments from shell No. 5 of Fig. 1. Samples are lettered from the edge (a) inwards to (n).

Mook and Vogel found that in each river, analyses plotted as our Fig. 1 fell near a straight line, the points indicating various mixtures of the sea water and river water end members. At first it seemed that our results (Fig. 1) displayed the same feature; that is to say, prehistoric man collected cockles at various points along a former estuary.

To investigate the matter further, successive growth increments were analysed in some of the shells. The result of this procedure for one shell (No. 5 in Fig. 1) is shown in Fig. 2. The points are lettered from the edge of the shell inwards. The procedure was as follows. The shell, length about 35 mm, was cut in half along a rib, and one edge was polished to reveal the growth laminae. The inner part of the shell, where growth layering parallel to the shell is visible, was filed away and discarded, after which samples weighing about 0.3 mg were taken with a triangular file at intervals of about 1 mm along the rib. This ensured that each sample represented a well defined growth interval. The samples were roasted at 450° C *in vacuo* for 30 min to remove organic matter, after which carbon dioxide was released by the action of 100 per cent orthophosphoric acid at 50° C (refs. 6 and 7). The gas was compared with a running standard using a double-collection mass spectrometer, and the results were calibrated by analysing standard carbonates under the same conditions. The overall reproducibility of the analyses during this series was about ± 0.15 parts per thousand.

It is clear from Fig. 2 that the shell lived in an extremely variable environment. Sample *n* implies more or less undiluted Mediterranean water, while sample *b* probably represents a mixture of about one part sea water to two parts river water. The variation is cyclic; our analyses extend through two complete years. This is confirmed by a visual examination of the shell, which shows an alternation of clean and dirty colour. The isotopic variation seems to be consistent with the Mediterranean climate, in which rainfall and therefore river flow are intensely seasonal. In the mountains of northern Greece, where the river Aliakmon rises, monthly rainfall in the dry months of July to September is only about 1½ inches, while in some winter months it may be ten times as much. Evidently this particular shell died, and presumably was eaten, in the spring. Sample *b*, not far from the edge, represents the lowest salinity of the previous winter. Samples *f* and *g* represent the previous summer, and so on (note that no special care was taken to space the samples equally, so that the distribution of points does not accurately represent the distribution of growth through the year).

In the light of Fig. 2, it now seems that Fig. 1 should be interpreted as representing variation in the season of

collection among the shells sampled, rather than (or as well as) variation of position of collection along an estuary. There seem to be at least three separate areas of knowledge into which this study extends.

First, the palaeoenvironment of the Neolithic occupation site is better understood. The water body near the site was evidently freely connected with the sea as an estuary. In summer, the water would have had the salinity of the open sea, while in winter the salinity was much reduced by river inflow. It may be interesting to examine the fish bone in the food refuse in the light of this knowledge. The water would have been inhabitable to certain species only in the summer, so that the presence or absence of such species may indicate whether the settlement was deserted in summer as cattle were taken to higher ground, or whether it was continually occupied.

Second, it seems that even in an estuarine environment, where the effect of temperature on the isotopic fractionation between water and carbonate is swamped by the much greater changes in the isotopic composition of the water itself, we are able to determine the season of the year in which a shell was collected. Clearly a greater number of samples, critically selected, must be analysed before a reliable assessment can be made of the proportion of the year during which the site was inhabited, and at this particular site we are limited by the fact that shellfish was not an important part of the diet in any case. Even so, the definite information provided will be of value.

Third, this may prove a valuable tool in the wider investigation of climatic change. At least in the Mediterranean region, where estuarine mixing is not complicated by tidal effects, it may prove possible to utilize seasonal changes in the isotopic composition of fossil estuarine mollusc shells to discover the extent to which the seasonal rainfall pattern of the region has altered during the Pleistocene. This could greatly improve our understanding of the Mediterranean pluvial phases which seem to have coincided with the glaciations in more northerly latitudes.

I thank Dr R. J. Rodden, director of the excavations at Nea Nikomedeia, for putting samples at my disposal for this study. I also thank Mr M. A. Hall, who performed most of the analyses presented.

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Dipole Effect on the Vapour Pressure of Aliphatic Polar Liquids

KIRKWOOD's theory¹ for the interaction of a dipole-containing dielectric sphere with a dielectric continuum is used here in estimating dipole and dielectric effects on vapour pressure *p*. There have been many general and approximate examinations of such effects, including, for instance, the relationship of permittivity with "internal pressure"², the effect of a dipole on the boiling point³ and, for mixtures, the effect of polarity on the excess functions⁴, but there have been none involving a direct comparison of prediction with observation.

We approximate the nonpolar dispersion interactions by equating these to the values for "isostructural" hydrocarbons obtained by summarily replacing⁵ N or O by CH₃. For example, for both acetone

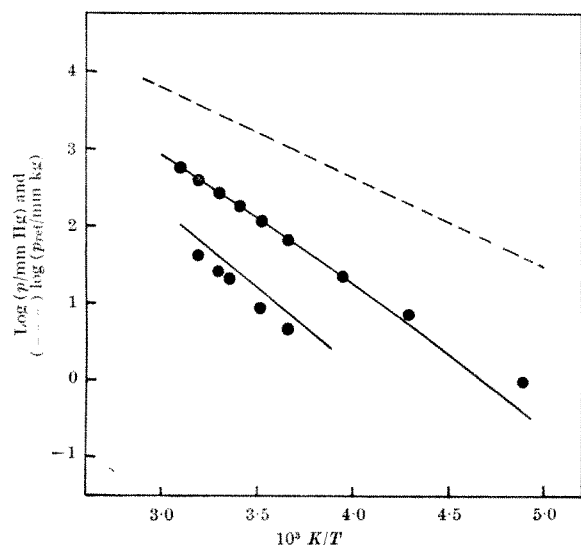


Fig. 1. Log p (observed are full lines, and calculated are filled circles) and log p_{ref} (isobutane, broken line) against inverse temperature. Acetone, upper line, -70 to 50°C . Nitromethane, lower line, 0 to 40°C .

and nitromethane we take isobutane as the nonpolar reference liquid. Seemingly anomalous polar liquids like ether (slightly more volatile than its reference liquid, pentane, contrary to what is expected for an apparently additional polar interaction) fall within our scope, which we have limited to liquids clearly suitable to being treated as continua. Such are the Onsager liquids, those for which the Onsager equation⁵ yields dipole moments from the liquid permittivities in good agreement (± 10 per cent) with the gas-phase values.

The appropriate Legendre function¹ yields for the work w_e of creating a dipole moment μ in a dielectric sphere,

volume v , of internal permittivity ϵ_i , surrounded by a dielectric continuum of permittivity ϵ ,

$$w_e = w_0 + \frac{4\pi\mu^2}{3v} \cdot \frac{\epsilon_i - \epsilon}{\epsilon_i(2\epsilon + \epsilon_i)} \quad (1)$$

(w_0 is the dielectric-independent self-energy term¹¹ which will be eliminated). For transfer of the sphere from vacuum to a dielectric of permittivity ϵ the work of immersion w is obtained by subtracting from (1) the corresponding expression with $\epsilon = 1$, giving

$$w = -\frac{4\pi\mu^2}{3v} \frac{\epsilon - 1}{(\epsilon_i + 2)(\epsilon_i + 2\epsilon)} \quad (2)$$

Assuming that the difference in chemical potential between a polar and corresponding nonpolar reference liquid arises only from electrostatic interactions which we equate with w , and neglecting non-ideality of vapours, we obtain for the respective vapour pressures p and p_{ref}

$$p = p_{ref} \cdot \exp(w/kT) \quad (3)$$

In (2) we take for v the molecular volume in the liquid, $M/\rho L$, and put $\epsilon_i = n^2$, n being the Na-D refractive index.

We test the equations for the well-documented cases⁶⁻¹⁰ of acetone and isobutane, and nitromethane and isobutane, for which data are available over extensive temperature ranges. Fig. 1 illustrates the satisfactory agreement between the values calculated from (3) (filled circles) and the observed p values (lines).

On the other hand, for ethers and tertiary amines (μ appreciable but ϵ small) equation (2) yields numerical values of $w/kT \ln(10)$ smaller than 0.1 . Such values are indistinguishable from zero in view of the approximations in our model, and log p for such polar liquids, when plotted against log p_{ref} , should thus yield a line of unit slope through the origin. This prediction from the Kirkwood equation is again quite satisfactorily substantiated (Fig. 2). The perceptible scatter here serves to illustrate the extent

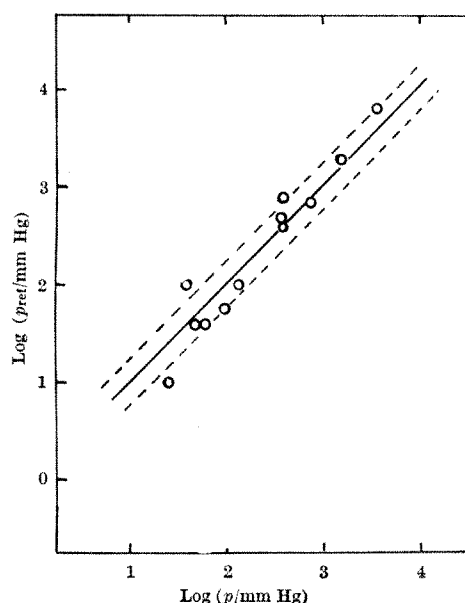


Fig. 2. Logarithmic plot of vapour pressure p of polar liquid against p_{ref} of reference liquid, for polar liquids having $w \sim 0$. Temperatures in the range -12 to 27°C , except for tripropylamine, at 157°C . From left to right, polar liquids having log $(p/\text{mm Hg})$ between:

1 and 2: 1,2-diethoxyethane, dioxane, triethylamine, dipropyl ether, di-isopropyl ether.

2 and 4: ethylpropyl ether, methylpropyl ether, diethyl ether, trimethylamine, tripropylamine, methyl ethyl ether, dimethyl ether.

[Broken lines are ± 0.25 units from full 45-degree line through (0,0).]

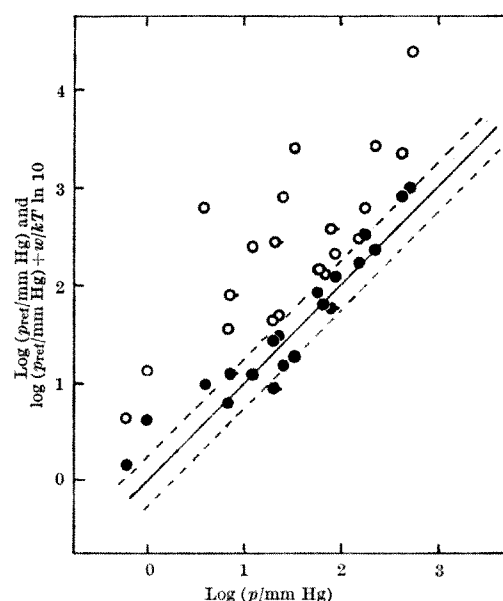


Fig. 3. Logarithmic plot of vapour pressure p of polar liquid against p_{ref} of reference liquid (open circles): also of p against calculated values (filled circles)⁸⁻⁹. Temperatures in range 10° – 30°C . From left to right, polar liquids having log $(p/\text{mm Hg})$ between:

-0.5 and 0 : 2-octanone, 2-heptanone.

0 and 1 : dimethylformamide, cyclohexanone, mesityl oxide.

1 and 2: 1-nitropropane, ethylpropionate, 2-nitropropane, n-propylacetate, nitroethane, nitromethane, methylpropionate, n-propylformate, 2-butanone, ethylacetate.

2 and 3: tetrahydrofuran, methylacetate, acetone, methylformate, acetaldehyde.

[Where necessary, tags identify associated filled and open circles. 45-degree line through (0,0), ± 0.25 , again shown.]

of approximation in our assumption for the nonpolar interactions.

Fig. 3 involves liquids for which $w/kT \ln 10$ is appreciable. For these, the dielectric and vapour pressure data are limited effectively to one temperature⁷⁻¹⁰ (dipole moments being often values in the solution-phase⁶), so a plot of the type of Fig. 2, rather than of Fig. 1, is presented. The reference-hydrocarbon vapour pressures p_{ref} , plotted logarithmically against polar-liquid values p , can be seen to have a wide scatter (open circles). But when $\log(p_{\text{ref}}) + w/kT \ln(10)$, representing calculated polar-liquid values, are plotted against $\log p$ (filled circles), the graph becomes again a distribution of points about the (0,0) line of unit slope, once more supporting equation (3).

Errors clearly arise from three main sources—the nonpolar-interaction assumption discussed with Fig. 2, experimental error in the data, and intrinsic inadequacies of the model. The second can be appreciable. McLellan⁶ noted that gas-phase μ values for a particular substance can be uncertain by as much as 0.2 D or more. Only part of the deviation occurring in Fig. 3 then arises from the model, as discussed in detail by Onsager⁵ and Kirkwood¹. The chief merit of the model as deployed here is its freedom from adjustable parameters.

Aromatic compounds fail to conform to the model, presumably because of breakdown from conjugation effects in the assumption of a nonpolar interaction. In this connexion compounds containing second-period and larger atoms have been omitted because measurements on the appropriate reference liquids are lacking. These exclusions apart, all Onsager liquids examined did conform.

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High Strength, High Modulus Graphite Fibres from Pitch

FIBRE composite technology has advanced rapidly in recent years with the development of several types of strong, stiff fibres, for example, S-glass, boron and graphite. The superior structural efficiency of graphite fibres is due to a combination of high strength and modulus and low specific gravity. The high modulus, and to some extent the high strength, is thought to be a consequence of the high degree of orientation and the small size of the graphite crystallites^{1,2}. To date such graphite fibres have only been obtained from synthetic, oriented, polymeric precursor fibres, of which the two main examples are rayon and polyacrylonitrile (PAN). In this laboratory high performance, highly oriented continuous graphite fibres have been produced from a number of readily available and inexpensive pitch precursors.

The crude pitch, for example, asphalt, is first refined by distillation and pitch fibres melt-spun onto spools. The fibres are then subjected to controlled oxidation and car-

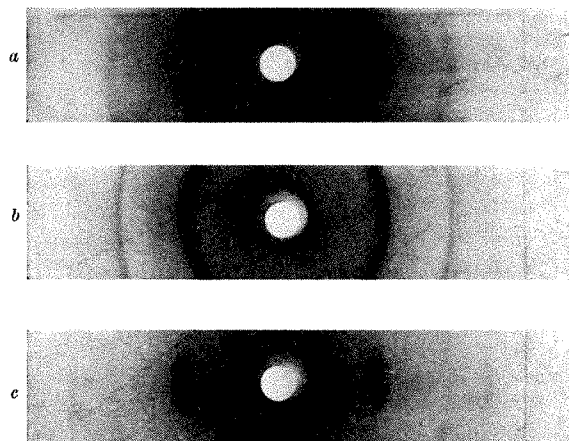


Fig. 1. Debye-Scherrer X-ray diffraction patterns of (a) carbonized (1,000° C) pitch fibres, (b) graphitized (2,500° C) fibres and (c) "stretch-graphitized" (2,500° C) fibres of average Young's modulus $\sim 38 \times 10^6$ pounds/inch².

bonization (to 1,000° C) similarly to methods previously used³. The resulting carbon fibres (representing a weight yield in excess of 65 per cent based on the refined pitch) are of uniform diameter (5–50 μm) and exhibit tensile strengths to 1.75 GN/m² (250,000 pounds/inch²) and Young's modulus in the range 20–50 GN/m² ($3\text{--}7 \times 10^6$ pounds/inch²). Their specific gravity is ~ 1.6 . Debye-Scherrer X-ray diffraction patterns of a fibre bundle (rotated axially) indicate that the carbon is isotropic and substantially amorphous (Fig. 1a). From the X-ray line broadening the crystallite dimensions are $L_c \sim 1 \text{ nm}$ (10 Å) and $L_a \sim 1.7 \text{ nm}$ (17 Å).

The production of highly oriented graphite from these carbon fibres requires carefully controlled elongation of the fibre during graphitization in the temperature range 2,000° to 2,800° C; to date uniform elongations as high as 140 per cent have been obtained on continuous fibres. The effect of the high temperature axial plastic deformation is preferentially to orient the crystallites, tending to align the graphite basal planes parallel to the fibre axis. Analogous effects have been observed in graphite fibres prepared from both rayon⁴ and PAN⁵, while a similar deformation-induced anisotropy in bulk glassy carbon has recently been reported⁶.

Figs. 1a, b and c show Debye-Scherrer X-ray diffraction patterns for carbon fibre, carbon fibre graphitized without elongation and "stretch-graphitized" fibre, respectively. The high degree of preferred orientation obtained by

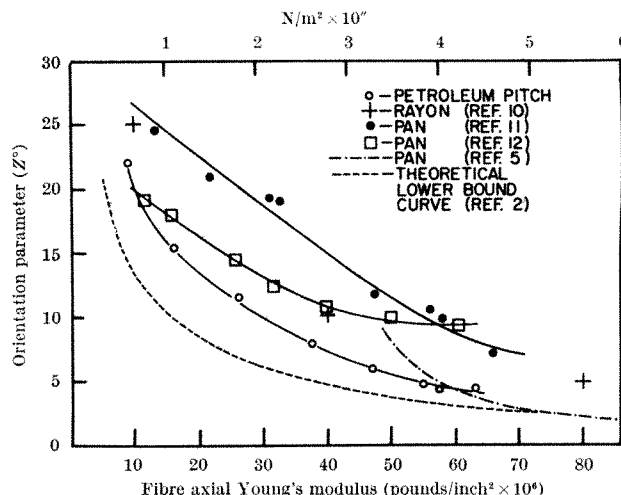


Fig. 2. Relation of the axial Young's modulus to the basal plane orientation in graphite fibres.

"stretch-graphitizing" these pitch carbon fibres is evident from the (002) arc length in Fig. 1c.

Fig. 2 shows the relationship between the axial Young's modulus of a number of graphite fibres, produced by "hot-stretching" to different elongations at $\sim 2,500^\circ\text{C}$, and the fibre preferred orientation. The orientation is represented by the angular half width, Z° , at half peak density round the X-ray (002) diffraction arc profile. A low value of Z° represents a pronounced degree of orientation. The modulus was measured by standard tensile test (Instron, 1 inch gauge length) and is uncorrected for porosity. Fibre diameters were measured using a shearing image eyepiece. For comparison the corresponding data available for graphite fibres produced from rayon and PAN, together with the theoretical lower bound curve for the homogeneous stress model², are included. To date, oriented graphite fibres have been produced from pitch precursors which exhibit strengths to 2.6 GN/m^2 ($375,000\text{ pounds/inch}^2$) and Young's modulus greater than 440 GN/m^2 ($70 \times 10^6\text{ pounds/inch}^2$). Bulk densities are similar to those of other graphite fibres.



Fig. 3. Bright field transmission electron micrograph of a thin longitudinal section of a "stretch graphitized", high modulus fibre ($\times c. 106,670$). Inset is the electron diffraction pattern from this area.

Transmission electron microscopy of a thin fibre section, cut on a microtome (Fig. 3), shows narrow elongated structural units lying parallel to the fibre axis. The electron diffraction pattern gives further evidence for the preferred orientation. This fibrous structure appears to be similar to that previously observed in rayon⁷ and PAN^{1,8} graphite fibres. For the latter structure an end-to-end stacked, turbostratic crystallite model, in which the individual crystallites are separated by grain boundaries and narrow voids, has been proposed^{8,9}. It is interesting to note that a similar microstructure is obtained in the present graphite fibres, derived from an essentially amorphous pitch, as is obtained in fibres produced from oriented polymeric precursors. Oriented, high modulus graphite fibres have also been produced recently from glassy carbon fibres made from thermosetting resins.

A more complete structure and property characterization of these fibres will be published at a later date.

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BIOLOGICAL SCIENCES

Quantitative Study of Cells reacting to Skin Allografts

THE cellular response to a strongly antigenic skin allograft is biphasic. Before rejection of the graft, cellular proliferation in the regional lymph nodes is largely confined to the intermediate (paracortical) zone. Then, at the time of onset of rejection, mitotic activity develops in the outer cortical zone and germinal centres are formed; simultaneously, or a little later, plasmacytes appear in the medulla of the nodes¹. The second phase is temporally, and probably also functionally, correlated with the production of humoral antibodies directed against antigens of the skin graft donor. The function of the first phase (during which antibodies are not normally detectable in the serum) is less clear, but is probably concerned with the development of the cellular mechanism for rejecting the graft. In broad terms, this cellular mechanism may be thought to consist of the production of "sensitized" lymphocytes, which become localized in the graft and there initiate a sequence of events which culminates in the graft's destruction. The meaning of "sensitization" in this context is uncertain, but some indirect evidence^{2,3} favours the possibility that the cells have on their surface specific antibody-like sites which are complementary to graft antigens. There is also one report of cells releasing haemolytic alloantibody at an early stage of the anti-graft reaction⁴. We have sought direct evidence for the existence of specific receptor-carrying cells by a modification of the cluster (rosette) technique^{5,6}.

Male and female CBA/H mice were grafted at 3–5 months of age with either allogeneic (A/H or A/Jax) or syngeneic skin from donors of the same sex. No differences between mice grafted with A/H and A/Jax skin, or between males and females, were detected, and the results were therefore pooled. Four to 27 days later recipients were killed. The brachial and axillary lymph nodes draining the graft area were removed and dissociated into a single-cell suspension, and the numbers of cells forming clusters with donor-strain and host-strain erythrocytes were determined for each mouse individually. Six million lymph node cells and 15×10^6 erythrocytes (both suspensions washed three times) were suspended together in 1 ml. of phosphate-buffered saline (pH 7.2), containing 5 per cent pooled CBA mouse serum, and were incubated overnight in siliconized 50×10 mm round-bottomed tubes at 6°C . During this time the cells settled. Half the cell-free supernatant was pipetted off, and the cells were resuspended by end-over-end rotation at 20 r.p.m. for 2 min. 0.05 or 0.1 ml. (approximately $3\text{--}6 \times 10^5$ cells) of each suspension was scanned at $\times 150$ magnification in a chamber made by supporting a 50×20 mm coverslip on a microscope slide with two narrow strips of polythene and 'Vaseline'. Possible clusters were verified at $\times 600$ magnification. The total number of nucleated cells remaining in the suspension was also determined. From these figures

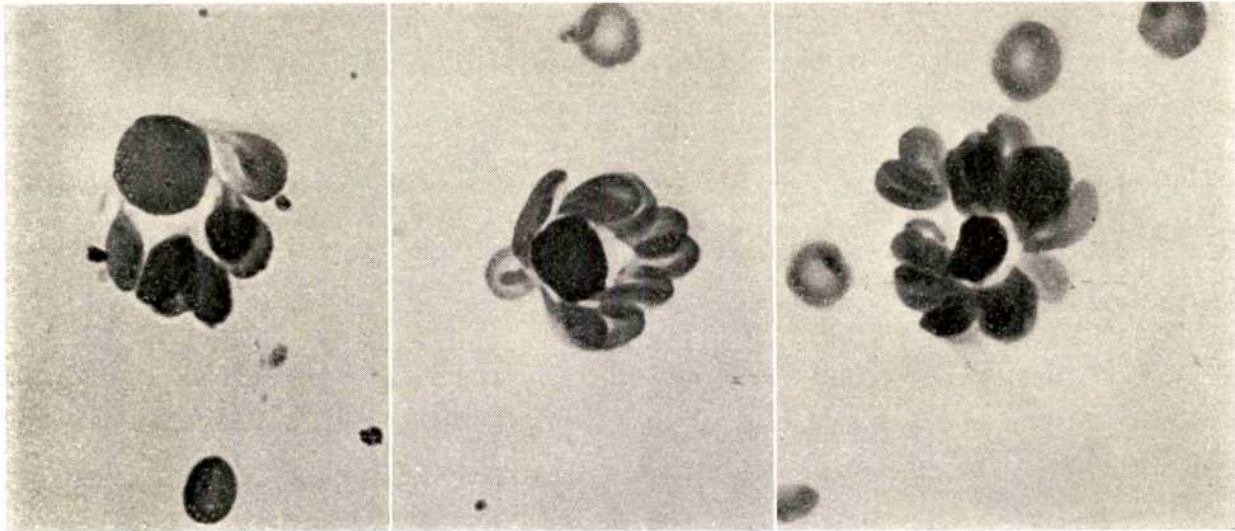


Fig. 1. Typical homoclusters as seen after air-drying, methanol fixation and staining with Leishman. $\times 1,200$.

the number of cluster-forming cells in the pair of lymph nodes was calculated. Three tightly adherent red cells were taken as the minimum criterion for a cluster, but most comprised between seven and fifteen (Fig. 1). Cluster-forming cells were placed in one of three categories according to their apparent diameter in the counting-chamber: $< 8 \mu\text{m}$, $8-11 \mu\text{m}$, $> 11 \mu\text{m}$. Because individual mouse sera vary in their capacity to support cluster formation, samples from three large pools, which had been tested and found to yield comparable results, were used throughout the series of experiments.

In accordance with the usual terminology of transplantation, clusters formed with erythrocytes of the same species are called homoclusters. This category is divided into autoclusters (erythrocytes from the same individual), synclusters (erythrocytes from another individual of the same inbred strain) and allocusters (erythrocytes from a different strain).

Allogeneic grafts broke down in 8-10 days while evoking a three-fold increase in the cellularity of the regional lymph nodes. Syngeneic grafts were accepted permanently, but also elicited some ($< \text{two-fold}$) increase in cellularity. Fig. 2 shows the numbers of allocuster and syncluster forming cells (AFC and SFC) in the nodes of allografted mice. A mean background of 67 AFC and 69 SFC was found in the lymph nodes of normal untreated mice. An exponential increase in the AFC count was seen between the fourth and twelfth days, followed by a slow return towards the background level from the fourteenth day onwards. At the earliest stage there was a concomitant rise in the number of SFC, but it was smaller and of shorter duration. The increase in the numbers of AFC and SFC in the regional lymph nodes is not the result merely of localization of such cells from elsewhere in the body, for they increase also in distant lymph nodes (unpublished data). No significant rise in either AFC or SFC was seen in syngrafted mice (Fig. 3). Most (67-88 per cent) SFC and "early" AFC had the light microscope morphology of small lymphocytes. Larger cells of $8-11 \mu\text{m}$ diameter (most of the AFC on days 10 and 11) had strongly basophilic cytoplasm. No clusters were observed around cells which appeared to be macrophages. It seems probable that skin allografts induce the formation of SFC by presenting the recipient with antigen(s) structurally related, but not identical, to "self" constituents. By analogy with the results following immunization of artificially tolerant animals with antigens related to the tolerogen⁷⁻¹⁰, some cells reacting against an allograft would be expected to cross-react to some extent with self constituents.

This would by no means rule out the direct involvement of cluster-forming cells in the rejection of the graft. The cluster assay gives no information about the energy of interaction (affinity) between lymphocyte and erythrocytes. This may be higher with allogeneic than with syngeneic (or autologous) erythrocytes (a situation analogous to that which occurs when, for example, bovine serum albumin (BSA)-tolerant rabbits are immunized with dinitrophenyl-BSA¹⁰), and this difference may be crucial *in vivo*, ensuring effective localization of the cells in the graft.

Alloantibodies were first detected in the serum 8 days after grafting (unpublished results of R. G. Kinsky, H. S. M. and N. A. S.). By this time there was definite evidence of cells reacting specifically with donor-type erythrocytes (Fig. 2), and it is probable that they were progenitors of antibody-secreting cells. It is not known whether earlier SFC or AFC released any antibody molecules. Hildemann's⁴ results suggest that some complement-fixing antibodies may be released several days before such antibodies are detectable in the serum. Their reactivity with autologous erythrocytes was not

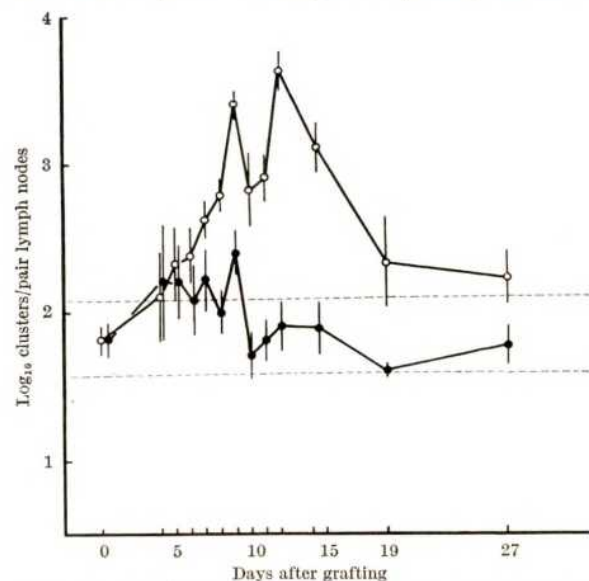


Fig. 2. Log_{10} numbers of AFC (\circ) and SFC (\bullet) in the draining lymph nodes of CBA mice grafted with A-strain skin (mean \pm s.e.). Broken lines indicate 95 per cent confidence limits for non-grafted controls. Points for day 0 are based on 28 mice, other points on 4-11 mice (median 6).

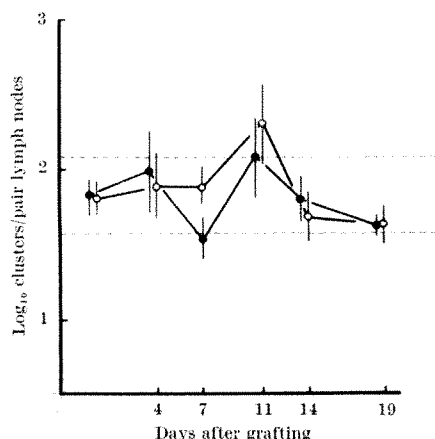


Fig. 3. Log₁₀ numbers of AFC (○) and SFC (●) in the draining lymph nodes of CBA mice grafted with syngeneic skin (mean ± s.e.). Broken lines indicate 95 per cent confidence limits for non-grafted controls. Points for day 0 are based on 28 mice, other points on 4–6 mice (median 6).

reported, but because their formation corresponded temporally with our SFC response they may have included autoantibodies.

It seems clear that the receptors on the lymphocyte surface for syngeneic or allogeneic erythrocytes are antibody-like molecules related to serum immunoglobulins, as has been shown for lymphocytic receptors for sheep erythrocytes¹¹. Elsewhere we present data in favour of this view, and discuss two possible functions of auto-reactive cells, one of which would give such cells a position of central importance in immunity^{12,13}. We also assume that AFC and SFC synthesize their own receptors and do not acquire them passively. The available evidence, especially the failure of other workers^{11,14–16} to demonstrate antibodies cytophilic for mouse lymphocytes, supports this view, but is not entirely conclusive.

Because an AFC/SFC response is detectable several days before rejection of a skin graft, the assay could possibly be adapted to give early warning of a rejection crisis in human transplant patients. Homocluster-forming cells are readily detectable in the peripheral blood of mice, although it remains to be seen whether the temporal pattern of response resembles that which is seen in the lymph nodes. Such a development would, however, depend on study of the effects of immunosuppressants on homocluster-forming cells and probably also on the use of nucleated target cells.

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Lymphocyte Stimulation in Pigs: Evidence for the Existence of a Single Major Histocompatibility Locus, PL-A

IN those species which have been sufficiently studied there is evidence of a single major histocompatibility locus (man, mouse, rat, chicken) that plays a predominant part in graft rejection. We present evidence for the existence of such a major locus in pigs by using the mixed lymphocyte reaction^{1,2}.

The technique of culture used has already been described³. 5 per cent inactivated foetal calf serum and 10 per cent inactivated weaner pig serum were added to the medium. Maximum stimulation occurred between 48 and 72 h with significant depression later. The incorporation of ¹²⁵I-labelled 5-iodo-2'-deoxyuridine was used to assess stimulation in the cultures⁴.

In the first experiment, seven piglets, A, B, C, D, E, G and I, of the same litter, were tested in one-way stimulation. The results of these experiments are represented in Fig. 1A,B. The litter of seven piglets can be divided into four groups of identical siblings as follows:



The probability of the mixed cultures being different from the controls by chance only was calculated and taken as significant at $P < 0.05$.

In the second experiment, a litter of eleven piglets was tested. To simplify the task, it was decided initially to use the two-way reaction; the results allowed a probable decision as to which were the incompatible piglets. The non-stimulations could be the result of compatibility, or to the inhibitory effect of the large number of cells used, the latter having already been reported^{1,5}. To explore this possibility, one-way stimulations were performed between some of the non-stimulating piglets (Fig. 1C,D). Some apparent non-reactors in the two-way system showed a good stimulation in the one-way system where smaller numbers of stimulating cells were used. The litter could therefore be represented as follows:



A slight stimulation was, however, found between C and E. The existence of antigenic differences in the minor histocompatibility loci could account for this stimulation, for a similar situation has been found between two human siblings whose HL-A typing showed identity (unpublished results of D. V. and R. Harris). This indicates that "strong" differences at minor loci can account for an MLC reaction in siblings identical at the major histocompatibility locus in some instances of coincidence, for example of several non-HL-A antigens. Such a situation has also been found in the mouse⁶. Some "strong" reactions between "weak stimulators" could also be explained in the same way. ("Strong" and "weak stimulators" are defined by the number of stimulating cells which are necessary to obtain maximum stimulation. With "strong stimulators" a low dose of stimulating cells is sufficient; higher doses inhibit the reactions.) Groups

having the double chromosome difference should exhibit strong stimulation; but this was not always the case. Members of the same group reacted in different ways, some being "strong" stimulators and some "weak" when tested against a piglet of a different group. Antigenic differences determined by the other minor histocompatibility loci, in addition to the major one, could account for these results. On the other hand, one chromosome difference could involve varying numbers of strong antigenic differences which could account for these "strong" stimulations. The quantitative differences in the reaction should therefore be interpreted with caution and only as a possible indication of a haploid or diploid difference.

Our findings provide evidence that in pigs there is a major histocompatibility system analogous to that of other species that have been studied. Because the pig is much used in experimental surgery⁷ and typing sera are not yet generally available, matching by the MLC reaction could be used to obtain compatible pairs of recipients for grafting experiments, which was the purpose of this work. The method could also be used to obtain typing sera by cross-immunization between siblings

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Studies on the HL-A Antigens of Human Lymphocytes: Inheritance in Clusters

A NUMBER of HL-A antigens are known to be present on human lymphocytes. We present here an interpretation of the genetic relationships between these antigens. The interpretation is based on an analysis of data collected in this laboratory.

Four hundred and twenty unrelated Australians were grouped by the cytotoxicity test for thirteen lymphocyte antigens. Not all of the subjects were tested for every antigen (Table 1). HL-A1, HL-A2, HL-A3, HL-A7, HL-A8, HL-A9 (LA4) and S Ly1 were each detected by a number of antisera so that the antigens could be identified beyond any reasonable doubt. Lc-20 (Walford) and S Ly2 were each identified by one antiserum only. HL-A5, 4a, 4b and 4c were difficult to define precisely and the reactions of each individual antiserum detecting these antigens were considered separately.

A computer was used to calculate the following parameters: antigen frequencies (Table 1), gene frequencies (Table 1), antigen associations (Fig. 1), and allelic relationships between the antigens. The gene frequencies (p) were calculated from the antigen frequencies (A) using the formula $p = 1 - \sqrt{1-A}$. The correlation coefficient (r), a measure of antigen association¹, was calculated using the formula¹ $r = \sqrt{\chi^2/n}$. The value of χ^2 was adjusted by Yates's correction.

The computer analysis helped to recognize allelic relationships between the antigens by compiling the frequency of cases in which the subjects were positive for three antigens. When this frequency was zero, no subject possessed more than two of the three antigens, indicating that the three antigens might be allelic to one another. None of the subjects tested was positive for more than two of the LA antigens or for more than two of the '4' antigens. Within the LA and '4' series the antigens were negatively associated with one another (Fig. 1). The LA and the '4' antigens seem therefore to be determined by two systems of multiple alleles.

Lc-20 was positively associated with HL-A1 and negatively associated with HL-A2, HL-A3 and HL-A9. S Ly2 was included in Lc-20. Consequently Lc-20 was related to the LA antigens. The 4a and 4b antigens were negatively associated with one another. These antigens are, however, complex and they may in fact be serological artefacts. S Ly1, HL-A5 and 4c seemed to be included in 4a, and HL-A7 and HL-A8 in 4b. Consequently 4a and 4b were related to the '4' antigens. The LA and '4' antigens were positively associated as follows: HL-A1 with HL-A8, HL-A2 with S Ly1, HL-A3 with HL-A7, S Ly2 with 4c. HL-A9, an LA antigen, seemed to be included in 4a, a '4' related antigen. HL-A1, an LA antigen, was nega-

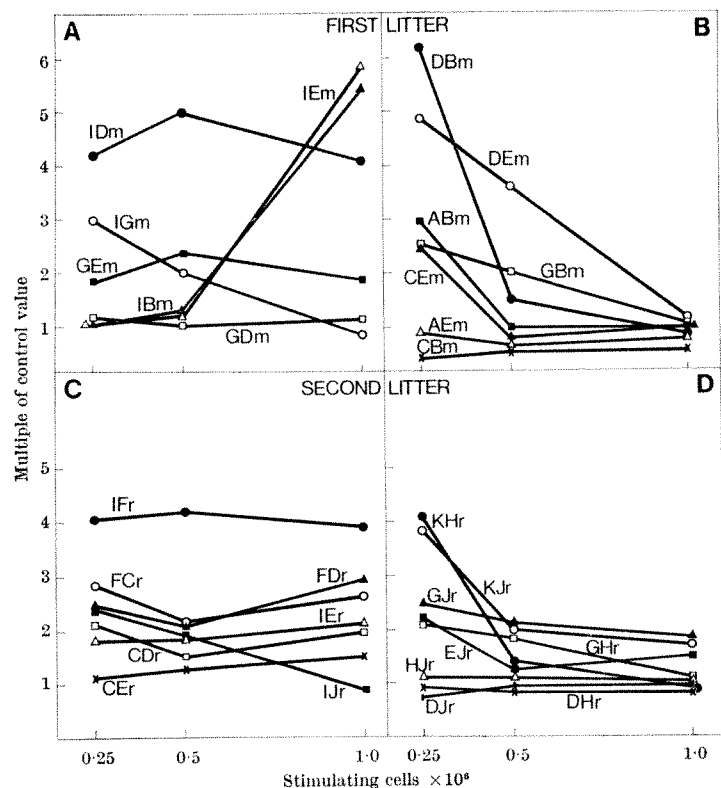


Fig. 1. One-way stimulation between piglets of the same litter. The stimulating cells were inactivated by mitomycin-C in the first litter and by X-rays in the second litter. The stimulation is expressed as multiples of the control, that is the sum of ¹²⁵I-iododeoxyuridine incorporation of both responding and stimulating (inactivated) cells which have been incubated separately for the same period of time as the mixed cultures.

having one haplotype difference. We suggest that the major histocompatibility locus in pigs should be called PL-A.

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Table 1. FREQUENCIES OF LYMPHOCYTE ANTIGENS AND GENES IN THE AUSTRALIAN POPULATION

Antigen series	Antigen	Antiserum	Antigen* frequency	Gene frequency	No. of subjects tested
LA	HL-A1		0.36	0.20	398
	HL-A2		0.51	0.30	400
	HL-A3		0.26	0.14	209
	HL-A9		0.18	0.09	187
	† S Ly2		0.10	0.05	348
Le-20	Le-20	Anderson	0.34	0.19	218
'4'	HL-A7		0.31	0.17	397
	HL-A8		0.21	0.11	278
	† S Ly1		0.27	0.15	363
	HL-A5	VR 2708	0.14	0.07	121
	HL-A5	To/01/02	0.19		130
4c		Fuller	0.26		340
	4c	Hamilton	0.28		106
	4a				
4a	4a	VR 1216	0.66	0.41	160
	4a	To/28/03	0.54		192
	4a	Kesler	0.49		256
4b	4b	Hinchcliffe	0.85	0.61	187
	4b	Normington	0.70		186
	4b	Brewer	0.31		203

* Or frequency of positive reactions of antiserum.

† S Ly indicates Sydney lymphocyte antigen; S Ly1 = KN-T12 (Kissmeyer-Nielsen).

tively associated with the '4' antigens, HL-A7 and S Ly1. HL-A2, an LA antigen, was negatively associated with 4b, a '4' related antigen. These associations were almost certainly a reflexion of the positive associations between the LA and '4' series already defined.

The concept of the LA and '4' series has been useful for defining the allelic relationships between the antigens within each series. But the statistically significant

associations and inclusions between the HL-A antigen series suggest that the antigens may be genetically determined not by two or more closely linked subloci, but rather by a series of allelic genes at the one chromosome locus. Each of these alleles could control several phenotypic expressions. For example, different alleles may determine each of the following pairs of antigens: HL-A1 and HL-A8, HL-A2 and S Ly1, HL-A3 and HL-A7, S Ly2 and 4c, possibly HL-A9 and a component of 4a.

The low power of tests of association in population studies¹ compared with family studies suggests that the former would detect only strong associations between antigens. The frequency of associations between the HL-A antigens in the Australian population (Fig. 1) implies that these antigens are strongly associated and reinforces the argument that they are inherited in clusters. This interpretation is analogous to Wiener's hypothesis² for the Rh system. Race and Sanger³ state that for the Rh system "we became embarrassed with hundreds of mutational sites" (regarded as subloci) "when previously we thought only three were needed". The Rh mutational sites are probably on the one gene rather than on a number of closely linked genes.

If the one gene has several mutational sites, the separation of antigens into allelic series could be the result of a series of mutations at the one position on the gene. Mutations at two or more such positions on the gene would give rise to two or more allelic series of antigens. If each gene determines an antigen in two or more allelic series, the antigens of these series would not be inherited separately but in clusters, as is so frequently seen with the HL-A antigen. Furthermore, no recombinations between

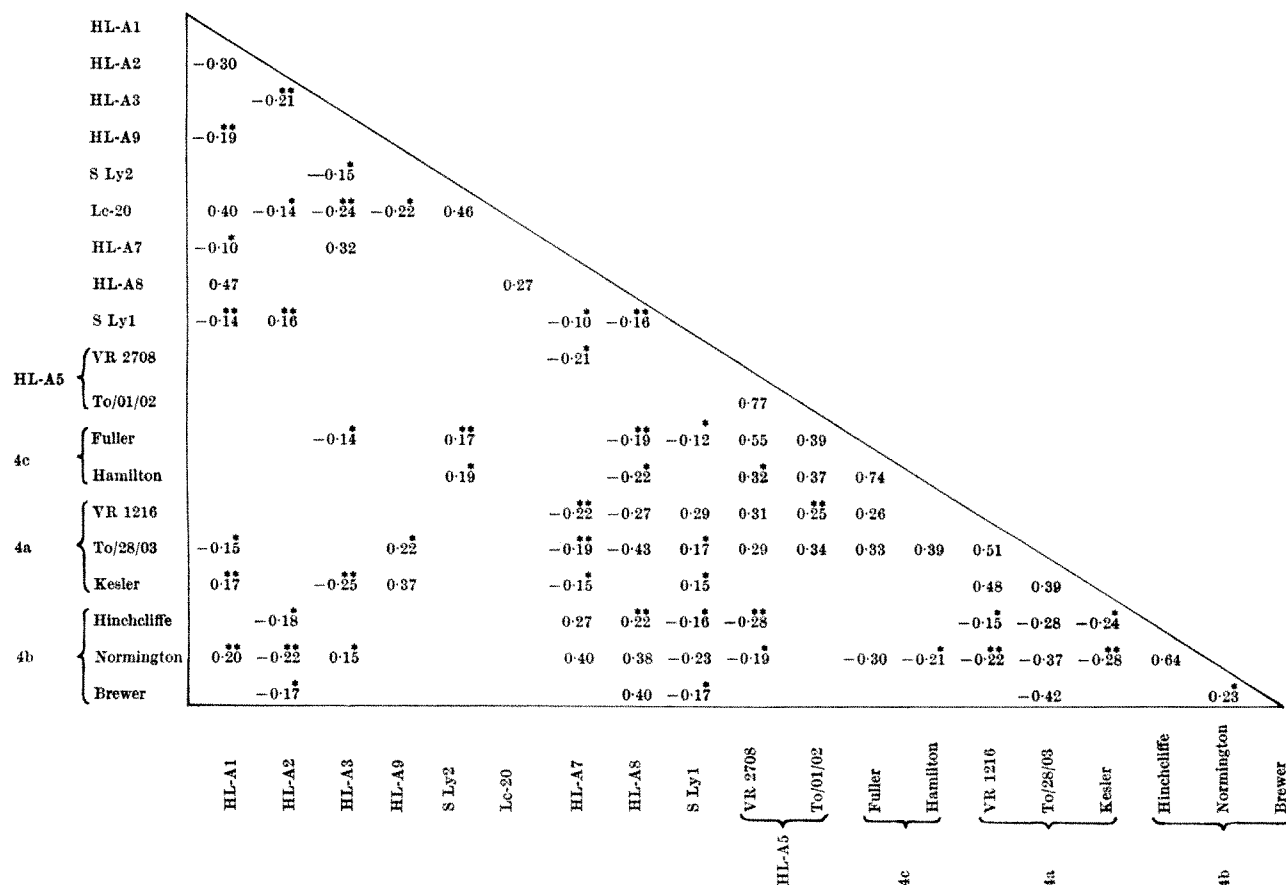


Fig. 1. Correlation coefficients between lymphocyte antigens (or antisera—see Table 1). The level of significance (P) of the correlation coefficient (r) is indicated as follows: $P > 0.05$: r is omitted from table; $0.05 \geq P > 0.01$: one asterisk above r ; $0.01 \geq P > 0.001$: two asterisks above r ; $0.001 \geq P$: no asterisk above r .

the antigens would be possible and indeed no absolutely convincing cases of recombinations between the Rh or the HL-A antigens have been reported.

The practical significance of this concept is that it predicts a number of genetically determined combinations of HL-A antigens. The recognition of these units of genetic information may facilitate HL-A antigen matching of recipients and unrelated donors before transplantation.

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Effect of 2-(α -Hydroxybenzyl)-benzimidazole and Guanidine on the Uncoating of Echovirus 12

2-(α -HYDROXYBENZYL)-benzimidazole (HBB) and guanidine have been shown to inhibit selectively the multiplication of picornaviruses¹. Both substances inhibit the appearance of a functional viral RNA polymerase², the replication of viral RNA^{3,4}, and the synthesis of viral coat protein⁴. While inhibition of synthesis of viral capsids seems to be a secondary phenomenon, probably a consequence of the failure of formation of viral RNA⁵, it is not clear whether the primary site of action of these compounds is principally the synthesis of viral RNA polymerase^{6,7}. As to the early steps of virus-cell interaction, it was shown that virus adsorption and penetration remain unaffected by HBB and guanidine^{4,8}, yet their effect on uncoating has not been determined. The period inhibitable by HBB and guanidine extends well into the exponential increase phase of the virus, thus covering the synthetic period of viral RNA polymerase and viral RNA, but it begins in the replication cycle at a time when synthesis of viral RNA polymerase and of viral RNA are not yet demonstrable^{3,4,8}.

Picornaviruses made photosensitive by incorporation of neutral red (NR) seemed to offer a possibility to investigate the uncoating process and whether it is affected by these inhibitors: previous studies on NR-poliovirus indicated that the dye is incorporated into the virus particle during virus multiplication; the virus particle thereby becomes photosensitive, but it loses its photosensitivity again after infecting cells⁹. This approach was taken in the following experiments.

Our test system consisted of echovirus prototype 12 (Travis) and primary rhesus monkey kidney cell cultures⁴. NR-echovirus 12 was prepared and its properties were determined. Echovirus 12 was grown for three passages in Eagle's minimal essential medium (MEM) containing 10 μ g/ml. NR. This operation, like any other involving NR-virus, was conducted under red light which was shown

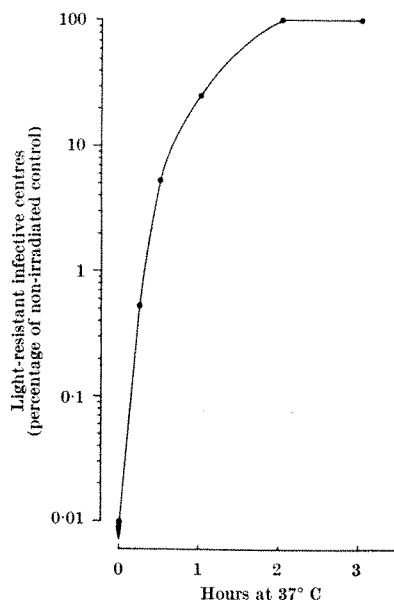


Fig. 1. Kinetics of uncoating of echovirus 12 after incubation at 37° C. Cell monolayers were infected with 0.1 p.f.u. per cell of NR-virus. After 30 min at 2° C, the cells were washed with cold phosphate-buffered saline, overlaid with Eagle's MEM and incubated at 37° C. At the times indicated, assays were made for total and for photoresistant infective centres¹⁰. The number of photoresistant infective centres is expressed as a percentage of the total amount in respective cultures. In all samples, the total number of infective centres per plate was about 1.1×10^5 . At time 0 (end of adsorption), only one photoresistant infective centre was found, that is, it amounted to less than 0.01 per cent of the total. The number of photoresistant infective centres then rapidly increased and reached a maximum by 2 h, here 100 per cent of the total.

to be non-inactivating. The titre of various preparations was in the order of $0.5-1.0 \times 10^9$ plaque forming units (p.f.u.)/ml. By irradiation with white light for 10 min¹⁰ the infectivity was reduced by more than 10^5 times. RNA extracted from NR-virus by phenol⁴ was found infective and was no longer photosensitive. If mixed with NR, the RNA lost its infectivity by exposure to light, but fully retained it in the dark. On the other hand, no infective RNA could be extracted from NR-virus previously exposed to white light. Exposure of standard virus (not grown in the presence of NR) to NR and white light had no effect on its infective titre. These observations and those obtained with poliovirus⁹ suggest that the dye is incorporated into echovirus 12, that its RNA is the target of photodynamic inactivation and that during uncoating desensitization takes place by dissociation of dye and viral RNA. Accordingly, in the following experiments loss of photosensitivity of NR-virus after it had infected cells was taken as a measure of uncoating. The kinetics of uncoating of echovirus 12 after incubation at 37° C is shown in Fig. 1.

In the following experiment the effect of HBB (D-2-(α -hydroxybenzyl)-benzimidazole hydrochloride)¹¹ and guanidine hydrochloride on the uncoating of echovirus 12 was tested. Cells were pretreated for 20 min with either 0.1 mM HBB, 2 mM guanidine, or phosphate-buffered saline, and were then infected. The test was carried out in principle as described earlier, except that in respective culture media the indicated compounds were present until irradiation with white light took place. The percentage of uncoated virus was determined after incubation for 3 h at 37° C, a time when uncoating had reached its maximum in untreated controls. In order to make sure that the compounds were in fact inhibitory to the virus, sets of cultures with or without the compound were incubated for 8 h after the end of adsorption. This time corresponds to a single cycle of virus multiplication⁴. After collection, the virus materials were irradiated with white light to destroy input virus and were then tested

Table 1. EFFECT OF HBB AND GUANIDINE ON UNCOATING OF ECHOVIRUS 12

Treatment	Exposure to light	Infective centres per plate 3 h at 37° C	Virus yield (p.f.u.) per plate 8 h at 37° C
Untreated control	—	2.7×10^5	
	+	2.3×10^5	1.1×10^8
HBB, 0.1 mM	—	2.5×10^5	
	+	2.1×10^5	$< 1.0 \times 10^1$
Guanidine, 2 mM	—	2.2×10^5	
	+	1.8×10^5	1.2×10^8

for the yield of newly synthesized virus. The results are given in Table 1.

Although HBB and guanidine strongly inhibited the multiplication of echovirus 12 (reduction of virus yield by 10^6 times and more), they had no effect on the uncoating of the virus, because the percentage of photoresistant infective centres was similar in both treated and untreated groups. These results are compatible with kinetic studies on the multiplication of various picornaviruses in the presence of the inhibitors^{4,8}, but because guanidine inhibits the development of ultraviolet-irradiation resistance by poliovirus infective centres¹²—a Luria-Latarjet type of experiment—an event immediately following uncoating must be affected by guanidine. Further studies will reveal the nature of these biochemical events.

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Crystal Structures of Dimers of Uracil and 6-Methyluracil

THERE is at present great interest in the chemical and biological effects of ultraviolet irradiation of nucleic acids. The possible correlation between the stereochemistry of the photoproducts and the biological consequences requires a detailed knowledge of the structures of these photo products. In addition to the methods of nuclear magnetic resonance^{1,2}, mass spectra³, topochemistry⁴⁻⁷ and chemical analysis⁸⁻¹⁰, X-ray analysis is most conclusive. Consequently, crystal structure analyses have been performed on an adduct of thymine¹¹⁻¹³ and a trimer of thymine (unpublished) in addition to the *cis-syn*¹⁴⁻¹⁶, *cis-anti*¹⁷ and *trans-anti*^{18,19} cyclobutyl dimers of uracil and thymine derivatives. The analyses of various dimers show remarkable agreement in bond lengths and angles of the molecules even though the packing and hydrogen bonding, if any, are quite different for the various materials. The *cis-syn* and *cis-anti* forms all have a puckered four-membered ring with a dihedral angle $\sim 155^\circ$ except for the *cis-syn* photoproduct from 1,1'-trimethylene bis-thymine where the trimethylene bridge seems to force the cyclobutyl ring into a planar configuration¹⁶. The two structure determinations of the *trans-anti* form showed that the molecules themselves have a centre of symmetry and consequently the four-membered rings must be planar.

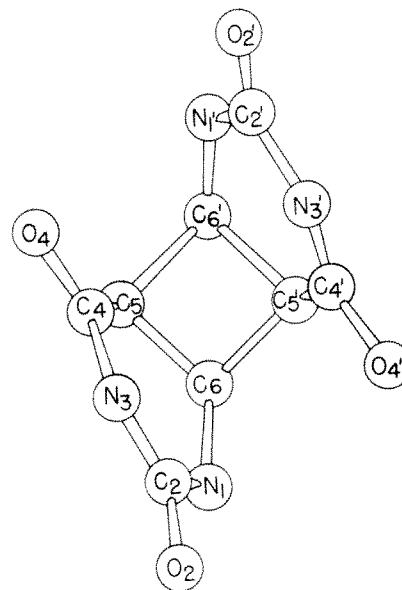


Fig. 1. The molecular structure of *cis-anti* U=U.

Two additional structure determinations are reported here. One is the *cis-syn* photodimer of 6-methyluracil crystallized with a molecule of water. It is unusual in that it is the first structure analysis of a hydrated form. The other is the *cis-anti* U=U, which results from the crystallization of U₃, a trimer of uracil²⁰. Apparently, U₃ has the same properties as the thymine trimer (T₃ or PT₃) (ref. 21), that is, some characteristics of the pyrimidine adducts, the photoreversibility of the pyrimidine dimers and the instability of a thymine photohydrate. But U₃ is much more unstable than the corresponding T₃. Although the structure of thymine phototrimer has now been established, attempts are being made to establish the structure of U₃.

To prepare *cis-syn*-6-methyluracil dimer (6MU=6MU), an aqueous solution of 6MU (2 mM; 5 l.) was irradiated in a frozen state for 2 h (ref. 22). In these conditions, 20 per cent dimerization was observed. After being thawed, the irradiated solution was evaporated until dry. The residue was taken up in a small volume of hot water and was applied to a 2 × 50 cm column of Dowex ('50W-X12', H⁺ form, 100-200 mesh). The column was eluted with distilled water. The first 200 ml. eluants having end absorption were combined as the "dimer fraction". This solution was concentrated under reduced pressure to 25 ml. and, after it had been allowed to stand at 5° C overnight, large colourless cubic crystals formed. This material was converted to 6MU on irradiation with 254 nm light in water. It is, however, stable in 2 M NaOH and in 6 M HCl. The *R_f* values of this compound are 0.38 (0.70 for 6MU) in *sec*-butanol and water (50:20); 0.25 (0.54) in *n*-butanol, acetic acid and water (80:12:30); 0.41 (0.64) in *tert*-butanol, methyl ethyl ketone, formic acid and water (40:30:15:15) and 0.40 (0.57) in *tert*-butanol, methyl ethyl ketone, concentrated ammonia and water (40:30:15:30) respectively.

cis-anti-Uracil dimer (U=U) was prepared according to the method reported previously for the U₃ (trimer of uracil) by the irradiation of uracil in ice²⁰. The conditions used for the crystallization of U₃ at boiling water temperature caused it to decompose to *cis-anti* U=U.

X-ray diffraction data for both crystals were collected photographically, along two axes, with the multiple-film, equi-inclination Weissenberg technique. Cell parameters and other data are listed in Table 1. Each structure was solved directly by the symbolic addition procedure for phase determination²³. Atomic coordinates

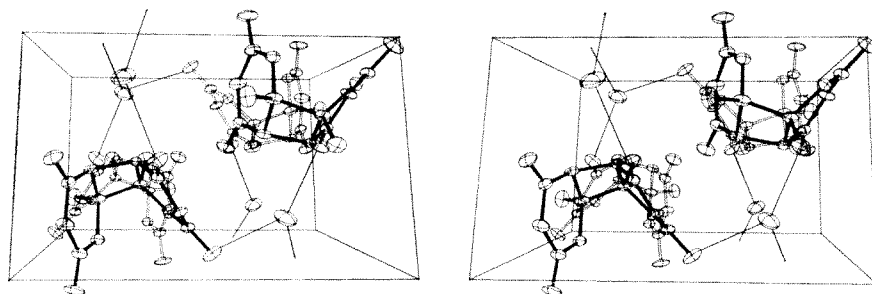


Fig. 2. The stereodiamgram of a unit cell of 6MU=6MU crystals.

(available on request) and anisotropic thermal factors were refined by least-squares methods and hydrogen atoms were located by means of difference maps. The agreement between observed and calculated structure factors is 9.9 and 10.6 per cent for the U=U and 6MU=6MU compounds, respectively.

The molecule of the *cis-anti* photodimer of uracil (Fig. 1) possesses very nearly a two-fold rotation axis, although none is required by the space group symmetry. The cyclobutane ring is puckered with a dihedral angle of 150°. The two uracil residues are rotated with respect to each other by 21° (torsion angles about the C5-C6' and C5'-C6 bonds). Each uracil residue has seven atoms which are coplanar to within 0.07 Å, while C6 and C6' are 0.38 and 0.35 Å out of the planes of the respective uracil residues. In every respect, the molecular geometry of the *cis-anti* dimer of uracil is very similar to that of the *cis-anti* dimer of 1,3-dimethylthymine¹⁷. The molecular environment in the crystal is different, however, in that the 1,3-dimethylthymine dimer cannot make any hydrogen bonds, whereas each dimer of uracil participates in eight NH...O intermolecular hydrogen bonds which range from 2.83 to 2.97 Å. The efficient hydrogen bonding scheme accounts for the high density, 1.70 g/cm³, in the crystal.

The *cis-syn* photodimer of 6-methyluracil with one molecule of water of crystallization can be compared with the anhydrous crystal of the *cis-syn* photodimer of uracil¹⁵. Again, bond lengths and bond angles in the two molecules are very similar. The cyclobutyl ring is puckered, but it is significantly flatter than in the anhydrous U=U. The dihedral angles in the cyclobutyl ring are 162° and the two uracil residues are rotated with respect to each other by 16.5° (torsion angles about C5-C5' and C6-C6') as compared with dihedral angles of 155° and torsional angles near 24° in the anhydrous crystal. Seven atoms in one of the uracil residues are coplanar within ±0.028 Å with C6 displaced 0.28 Å out of the plane, comparable with the configuration in the *cis-anti* U=U. The other uracil residue is composed of two planar units with a fold along the N3'...N6' line having a dihedral angle of 168.5°. As observed previously, there is little difference in the bond lengths and angles in the various photodimers which have been studied, but subtle differences in the skewness of the cyclobutyl ring and the deviations from planarity in the uracil or thymine residues are observed.

The fact that the crystals of 6MU=6MU contain the water molecules may be particularly interesting because it may correlate with the anomalous spectral data in

nuclear magnetic resonance and infrared for this photodimer (unpublished results). The stereodiamgram in Fig. 2 shows the contents of one unit cell. It is apparent that the dimers align themselves in rows in such a manner as to create continuous channels (along the *a* direction of the unit cell) for the water molecules. Each water molecule is hydrogen bonded to three different dimer molecules forming two OH...O bonds at 2.84 and 3.01 Å and one O...NH bond at 2.90 Å.

The four dimer molecules illustrated do not form hydrogen bonds with each other but they do form NH...O bonds with dimer molecules in adjacent cells.

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Table 1. CRYSTALLOGRAPHIC DATA FOR *cis-anti* U=U AND *cis-syn* 6MU=6MU·H₂O

Space group	<i>cis-anti</i> U=U P1	<i>cis-syn</i> 6MU=6MU·H ₂ O P2 ₁ /a
a	8.594±0.004	15.58±0.03
b	7.478±0.004	11.33±0.02
c	6.915±0.004	7.28±0.02
α	96.9°	90°
β	95.4°	107.2°
γ	85.5°	90°
Molecules/unit cell	2	4
Density (calc.)	1.704 g/cm ³	1.462 g/cm ³
No. of independent reflexions	1,453	2,139

Is Retinal-Phosphatidyl Ethanolamine the Chromophore of Rhodopsin?

SEVERAL laboratories have reported the presence of the Schiff base of retinal and phosphatidyl ethanolamine (retinal-PE) in bovine rod outer segments¹⁻³. Poincelot *et al.*³ claim that this compound is the chromophore of rhodopsin. Heller found only traces of phospholipid in purified bovine rhodopsin⁴, however, and presented evidence that retinal is attached directly to opsin in a substituted

aldimine linkage⁵. Hall and Bacharach⁶ injected frogs with ³²P and found large amounts of radioactivity in rod phospholipids, but none in purified rhodopsin. The purified rhodopsins of Heller^{4,5} and Hall and Bacharach⁶ had an absorption maximum at 500 nm and could be bleached by light. We recently determined the molar concentration of retinal-PE in bovine rods and compared it with the molar concentration of rhodopsin. We present here our preliminary findings.

Bovine rods were prepared by the common flotation-sedimentation centrifugation procedure using 37 per cent sucrose in 0.067 M phosphate buffer (pH 7.0). The rods were lyophilized and stored at -20° C in a light-tight desiccated container. Two preparations of rods were used in this study and are designated ROS 1 and ROS 2.

A known weight of dry rods was taken up in a known volume of 2 per cent aqueous digitonin and the spectrum between 240 nm and 650 nm was recorded. Absorbance at 500 nm was determined from the difference spectrum, and the molar concentration of rhodopsin was calculated from Beer's law ($A = \epsilon \cdot c \cdot l$). The data of Table 1 are in μ moles of rhodopsin per mg dry rods, calculated using molar extinction coefficients of Wald and Brown⁷ (40,600) and Heller⁴ (23,100). Differences in the rhodopsin concentrations for the two preparations can be explained by a higher concentration of protein in ROS 1. The A_{280}/A_{500} ratio was 4 in ROS 1 and 2.7 in ROS 2.

Phospholipids were extracted in the dark from known weights of dry bovine rods with anhydrous chloroform:methanol (2:1) made $10^{-3.5}$ M with anhydrous HCl gas. (Only about half of the lipid-phosphorus is removed from rods if chloroform is omitted from the extraction solvent. According to Poincelot *et al.*², no imine exchange occurs in model Schiff bases extracted in methanol made $10^{-3.5}$ M with HCl gas so that none should occur during this extraction.)

The retinal-PE content of the extracts was determined by two different procedures. In the first, the phospholipids while still in the extracting solvent were immediately hydrogenated over platinum oxide, converting the unstable Schiff base to a stable secondary amine. The reduced retinal-PE was separated from the other phospholipids by thin-layer chromatography and its phosphorus content compared with that of the total phospholipids. From the known molar concentration of phospholipids in the rods, the amount of retinal-PE (μ moles/mg dry rods) was calculated (Table 1).

In the second procedure, the rods were extracted in a known volume of the acidic solvent. The insoluble proteins were sedimented by centrifugation and the clear supernatant was removed and scanned. The molar extinction coefficient of synthetic retinal-PE had been previously determined to be 31,300, and this value was used to calculate the molar concentration of retinal-PE in the rods from Beer's law.

Table 1. CONCENTRATION OF RETINAL-PE AND RHODOPSIN IN BOVINE ROD OUTER SEGMENTS

Sample	Rhodopsin μ moles/mg dry rods		Retinal-PE μ moles/mg dry rods	
	ϵ (Wald)	ϵ (Heller)	P assay	Spectral assay
ROS 1	3.1×10^{-3}	5.4×10^{-3}	2.5×10^{-3}	2.7×10^{-3}
ROS 2	4.3×10^{-3}	7.5×10^{-3}	1.5×10^{-3}	1.9×10^{-3}

The similarity in the values for the retinal-PE content determined by the two procedures indicates that the numbers in the table are accurate estimates of the content of this lipid in bovine rods. Clearly the molar concentration of retinal-PE in bovine rods is not as high as that of rhodopsin, and it is therefore doubtful that it serves as the chromophore of rhodopsin, as claimed by Poincelot *et al.*². We are not suggesting that retinal-PE does not participate in the visual process. Its concentration in rods is such that it could serve to store 11-*cis*-retinal and function as a retinal "donor" in the regeneration of rhodopsin. This is now being tested.

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A "Deep Sea Rhodopsin" in a Mammal

In a broad sense the absorbance spectra of the rhodopsins of fishes seem to vary to suit the spectral energy distributions of the lights reaching them through the water¹⁻⁶. Thus bathypelagic fishes, living in an environment where the ambient daylight (and the light from bioluminescent organisms) is maximal between 460 and 490 nm⁷, have rhodopsins of λ_{\max} between 472 and 487 nm (refs. 1-5). Similarly, coastal fishes, which live in light environments with peak radiation between 490 and 550 nm⁷, have rhodopsins that range correspondingly in λ_{\max} between 495 and 512 nm (refs. 3-5, 8).

It interested us to know whether aquatic mammals showed similar modifications, and Dr J. Everson of the British Antarctic Survey was fortunately able to obtain for us the eyes of an elephant seal *Mirounga leonina* Linn. and a Weddell seal *Leptonychotes weddelli* (Lesson).

The two seals were shot near the Survey Base (Signy Island) on moonless nights in June (elephant seal) and October (Weddell seal) of 1965. The eyes were removed at the Base and stored there in darkness in a deep-freeze until they were eventually sent to the Institute of Ophthalmology, London, where we processed them for their visual pigments in March 1969.

The retinas, one from each animal, were first washed in pH 7.0 buffer and then shaken with 2 ml. portions of 35 per cent sucrose solution to obtain suspensions of the photoreceptor outer limbs⁹. This procedure was repeated until no further yields of receptors were obtained, four suspensions being necessary for the elephant seal's retina and six for that of the Weddell seal. After precipitation of the photoreceptors they were bulked, dried in a vacuum desiccator and treated with 3 ml. portions of petroleum ether (b.p. 50°-60° C) until no more yellow pigment was extracted (nine extractions for the elephant seal and seven for the Weddell seal). The visual pigments were then extracted, each with 1 ml. of 3 per cent digitonin solutions. The yields of pigments from the two species were similar, that from the elephant seal having an absorbance maximum (1 cm path) of 0.96 (at about 485 nm) and that from the Weddell seal a maximum of 0.94 (at about 495 nm). Re-extraction of the residues with further 1 ml. portions of the digitonin solution yielded only a little more pigment.

Samples of the two extracts, after suitable dilution and addition of hydroxylamine, were examined by the partial bleaching technique (two bleachings at 600 nm, and a terminal bleach with "white" light filtered through a Wratten No. 15 filter). Both species were found to have

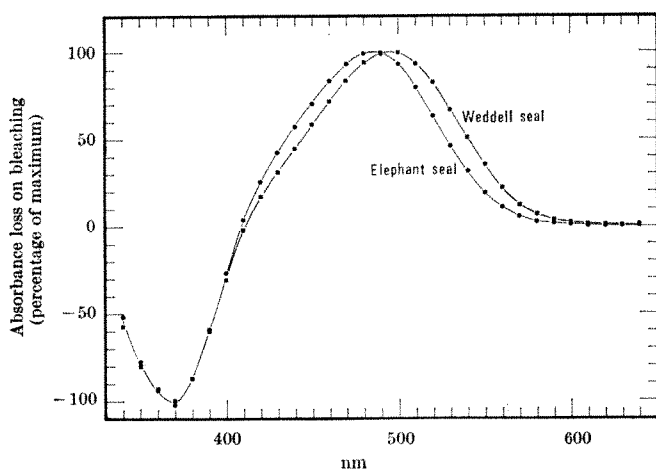


Fig. 1. Difference spectra of the two seal pigments in the presence of 0.02 M hydroxylamine (actual ΔA_{\max} = 0.49 (elephant seal) and 0.45 (Weddell seal) pH 8.6). Note that λ_{\max} for the elephant seal pigment is 485–486 nm ("deep sea rhodopsin") and that for the Weddell seal is 495–496 nm ("terrestrial and coastal water rhodopsin"). The product band in both cases (λ_{\max} = about 370 nm) is characteristic of the oxime of retinal.

essentially homogeneous rhodopsins with λ_{\max} = 495–496 nm in the Weddell seal, and λ_{\max} = 485–486 nm in the elephant seal (Fig. 1). Thus the rhodopsin of the Weddell seal resembles those typically found in coastal water fishes and terrestrial mammals, while the rhodopsin of the elephant seal resembles those of deep-sea fishes.

The Weddell seal is a south Antarctic species that lives near the coast or ice. The caval sphincter (that on the posterior vena cava, anterior to the diaphragm), which is associated with the diving habit in mammals, is not particularly well developed in this species¹⁰. Its teeth—and stomach contents—indicate that it feeds chiefly on fishes that are not bioluminescent¹¹.

The elephant seal, on the other hand, has a sub-Antarctic distribution and, save for the breeding period, lives a pelagic life (about which little is known) in deeper water¹². Judged by its very powerful caval sphincter it is capable of diving to great depths¹⁰. Its teeth, and stomach contents, suggest that it feeds on squids¹².

Thus, superficially at least, there seems to be a correlation between the ecology of these seals and their rhodopsins. In spite of its weak caval sphincter, however, the Weddell seal often dives 200 m or more into the exceptionally clear waters beneath the Antarctic ice¹³. Many pelagic fishes that are caught at comparable depths have "deep sea rhodopsins" but, as we have seen, the Weddell seal has not. This makes it reasonable to speculate that the "deep sea rhodopsin" of the elephant seal is an adaptation not so much to the ambient light of solar origin as to that emitted by the bioluminescent squids on which it feeds.

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Reversible Visual Pigment Changes in Tadpoles exposed to Light and Darkness

THE visual pigments of many amphibian larvae are mixtures of rhodopsin and porphyropsin, the latter usually, but not always, predominating¹⁻³. During the metamorphic climax, the porphyropsin is wholly discarded and the rhodopsin emerges as the characteristic pigment of the adult retina. I describe here a hitherto unsuspected reaction of the larval retina: as in fishes⁴⁻⁸, the balance between rhodopsin and porphyropsin can be altered by exposure to light and darkness. The phenomenon does not seem to be connected with the metamorphic process.

Fig. 1 illustrates difference spectra of the visual pigments extracted from *Rana pipiens sphenocephala* tadpoles. These animals started out with 39 per cent porphyropsin and 61 per cent rhodopsin. The difference spectrum of this mixture is shown by the dashed curve. A group kept in darkness for 26 days had a blue-displaced spectrum representing pure rhodopsin, while another group kept in continuous light over the same period had a red-displaced spectrum representing as much as 69 per cent porphyropsin. The system differs from fishes, where darkness favours porphyropsin while light favours rhodopsin⁴⁻⁸.

In this experiment, there was no difference between the metamorphic levels attained by the light and dark groups, even though the latter lacked the larval visual pigment. Moreover, another experiment with *R. catesbeiana* tadpoles revealed that, unlike the results of metamorphosis, the effects of light and darkness are reversible. In this respect the situation resembles that observed in fishes. Initially, the tadpoles (average Taylor-Kollros⁹ stage X) had 84 per cent porphyropsin (compare Wilt¹⁰). They were divided into two groups. Group A was placed in a light-tight cabinet, group B under continuous illumination. After 1 month the porphyropsin of group B had remained almost unchanged at 88 per cent, but had dropped to 40 per cent in group A (Fig. 2). The light conditions were then reversed. Group B was transferred to the dark, when the porphyropsin diminished to 44 per cent after 1 month; and group A was transferred to the light, when the visual pigments reverted to 88 per cent porphyropsin and 12 per cent rhodopsin. At all times, the average Taylor-Kollros stages of the tadpoles sampled lay between IX and XI.

Apparently, prolongation of the period spent in light or darkness did not induce any further shifts of pigment composition (Fig. 2). Preliminary analyses have indicated,

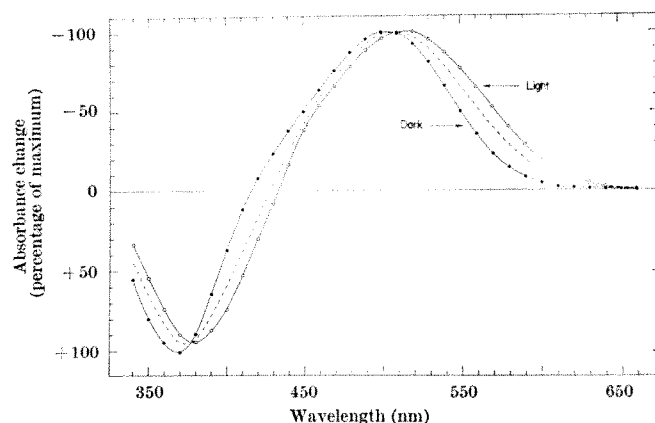


Fig. 1. Difference spectra of visual pigments from *Rana pipiens sphenocephala* tadpoles kept in light and darkness. Extracts in digitonin were prepared from whole eyes, and contained 10 mM hydroxylamine at pH 7. ---, Twelve specimens from initial batch; ○, eleven specimens kept unfed for 26 days under continuous illumination from a tungsten lamp (186 ft candles at water surface); ●, ten specimens kept unfed in complete darkness for the same period of time. The tadpoles originated from the Carolina Biological Supply Co. (July 1969).

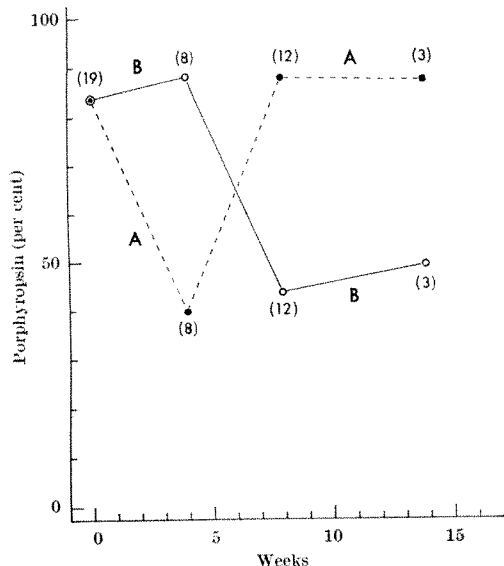


Fig. 2. Visual pigment changes in *R. catesbeiana* tadpoles kept in light and darkness. Group A (●—●) were placed in darkness for 4 weeks, then switched to continuous illumination (186 foot candles at the water surface); conversely, group B (○—○) were placed initially under continuous illumination for 4 weeks, then switched to darkness. Figures in parentheses represent the numbers of tadpoles in each sample. The tadpoles originated from the Carolina Biological Supply Co. (October, 1969).

however, that there is marked individual variation in the "dark" animals (but not in the "light" ones), so there is some uncertainty in this result which is based on only three specimens.

Is the effect under endocrine control? Thyroxine induces a shift towards porphyropsin in some salmonids¹¹, but it is not clear whether this hormone is responsible for visual pigment changes caused by light and darkness^{7,8}. In amphibian larvae thyroxine has the reverse action^{10,12}, so that one explanation for these observations would be an increase in thyroid activity in darkness. Alternatively, darkness might decrease the output of prolactin, a general antagonist of thyroid hormone during amphibian development¹³. An increase in thyroid activity (or decrease in prolactin) sufficient to alter retinal pigment composition should, however, be accompanied by accelerated and irreversible metamorphosis^{10,12}, yet the "dark" tadpoles were no further advanced than the "light" ones. This indicates that the light and dark-induced visual pigment shifts were not mediated by thyroxine.

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Adaptation in the Isolated Rat Retina

THE isolated retina is a useful preparation for studying many aspects of visual sensitivity. Photochemical changes following exposure to light may be studied directly, and concomitant electrical events may be monitored with gross and micro-electrodes. The photopigment is also not appreciably regenerated during an experiment, and loss of sensitivity following light exposure shows a clear relationship to visual pigment concentration.

Dowling¹ has shown a relationship between rhodopsin concentration and the logarithm of visual sensitivity by using a constant amplitude ERG response as a measure of retinal sensitivity. Rushton²⁻⁵, using psychophysical methods and reflective densitometry, has demonstrated the relationship in man. This has also been confirmed in the isolated rat retina⁶. Donner and Reuter^{7,8} have used ganglion cell responses as a criterion of retinal sensitivity, and they have demonstrated photopigment regeneration in this preparation. They have also related the rate of regeneration of rhodopsin to the recovery of sensitivity.

Baumann⁹ studied the relationship between the ERG and rhodopsin concentration in the dark-adapted isolated frog retina, and also found a linear relationship between log sensitivity and rhodopsin concentration. Baumann and Scheibner¹⁰ showed, however, that this relationship was non-linear when ganglion cell, rather than ERG responses, were used to determine sensitivity. They emphasized that the spatial characteristics of the stimulus might be responsible, in part, for this discrepancy, because large test spots may depress sensitivity because of lateral inhibition which affects ganglion cell, but not ERG, sensitivity.

Using large spot stimuli, we have shown¹¹ that there is a linear relationship between ERG and ganglion cell sensitivity. But Brown and Rojas¹² have shown that the rat retina contains two types of ganglion cell receptive fields: those having centres with antagonistic surrounds, and those without surrounds. Brown¹³ has tentatively related two specific dendritic configurations to these functional types. To find out if our earlier findings relating ERG and ganglion cell sensitivity need to be reinterpreted, we have repeated these experiments using various stimulus conditions.

The techniques used for isolating the rat retina and the perfusing medium have already been described^{6,11}. Rats were sometimes anaesthetized with intraperitoneal 'Nembutal', but about half of the experiments were conducted following decapitation without prior anaesthesia. In both cases, stimulus-induced responses were unaffected, with fairly constant thresholds from unit to unit. But 'Nembutal' tended to reduce the frequency with which spontaneously active units were found. An insulated platinum-iridium micro-electrode was introduced through a dual channel perfusion chamber from above, using a micro-manipulator. Electrode tips were 0.5–1.0 μ m in diameter. Impedance in saline was 100–500 k Ω at 1 kHz. The retina, illuminated from above, was mounted with receptors up, and the stimuli were presented from below. Electrical responses were led from a cathode-follower pre-amplifier to two Tektronix 122 amplifiers. One amplifier was set to a band width of 801 kHz, and the other to 0.2–20 Hz, to record the ganglion cell and the ERG responses, respectively. The amplifiers were connected to separate channels of a four-beam Tektronix 564 storage tube oscilloscope. Another beam displayed the output of a photocell which indicated the stimulus. A Polaroid camera was used to photograph the responses and ganglion cell responses were monitored with a Grass audio amplifier and loudspeaker.

The optical stimulator used a high pressure xenon arc lamp (Bausch and Lomb) with a regulated power supply. The light from this source was condensed and then collimated with a series of lenses. An electromagnetic

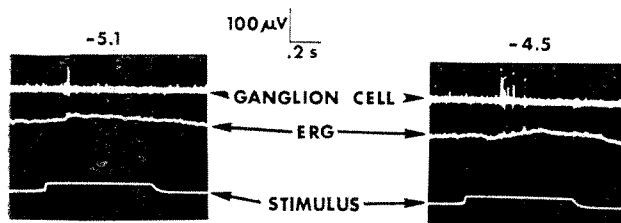


Fig. 1. Two oscillograms showing simultaneously recorded ERG and ganglion cell responses. The tracings on the left show a single ganglion cell action potential in response to the stimulus (on cell). The tracings on the right show responses to a stimulus 0.6 log units brighter than that on the left. Several action potentials are shown, but the ERG threshold has not yet been reached.

shutter ('Uniblitz') was used to present stimuli of 0.5 s duration. Repetitive stimulation could be presented with the aid of a Tektronix 161 waveform generator, 162 pulse generator, and 160 power supply, synchronized to trigger. Metal film neutral density filters (Optics Technology) and a pair of circular wedge filters (Kodak) mounted on a Gebhardt holder were used to attenuate the beam. Small spots and annuli could be presented to the retina by interposing two plastic disks with various sized apertures and black dots. These disks were attached to a calibrated mechanical stage with vernier scale, which permitted movements in the plane projected to the retina. The spot was $100 \pm 20 \mu\text{m}$, as measured at the retina. Another similar optical channel with a tungsten source enabled a second small spot of light to be projected on the retina. This second channel, combined with the first at a beam splitter, was also used to present large spot stimuli for ERG responses.

Thresholds for ERG and ganglion cell action potentials were determined in conditions of full dark adaptation, and also after 10 min of dark adaptation following 5 min exposures to light of increasing intensities. Usually three or four separate exposures to the adapting light were given, each of bleaching intensity⁶. The last and brightest light adaptation was repeated to ascertain that bleaching was, in fact, complete, with no further changes in sensitivity. Most experiments took 2–3 h.

Single ganglion cells were usually encountered after several random prings with the micro-electrode. This was advanced slowly through the retinal thickness with the aid of a micromanipulator, while presenting intermittent, dim stimuli. When expanded on the horizontal (time) axis, the uniform action potentials were almost always biphasic, indicating that the responses were being recorded extracellularly from the ganglion cell body.

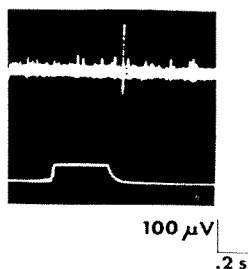


Fig. 2. Ganglion cell action potential in response to cessation of light stimulus (off cell).

The range of adaptation for ganglion cell responses in these experiments was 6.5 ± 0.5 log units. The thresholds for ERG *b*-waves of 10–25 μV were slightly higher (0.5–0.8 log units). Usually a single action potential was used as the criterion for the ganglion cell threshold, while a constant amplitude *b*-wave was used for the ERG threshold. Typical ganglion cell responses for both 'on' and 'off' cells are shown in Figs. 1 and 2.

In a typical experiment, there was good agreement between ganglion sensitivities obtained with both large

and small stimuli. Data combined from six experiments are plotted in Fig. 3. Here, two lines are illustrated, each providing a best fit for large spot and small spot data. The difference in slopes of these two lines is probably negligible.

Table 1. RECEPTIVE FIELDS OF RAT RETINAL GANGLION CELLS

	On-centre	Off-centre	Units with surrounds	Units without surrounds
Living rat (Brown and Rojas ¹²)	23	28	23	28
Isolated retina (present study)	25	5	1*	30

* On/off unit.

Most ganglion cells encountered were of the 'on' type. All except one had no surround. These results are at variance with those obtained from the living rat by Brown and Rojas¹² (Table 1). The data were obtained by determining threshold sensitivities at varying distances from the centre of the field (point of maximum sensitivity). The progressively decreasing sensitivity away from the centre is probably caused by scatter of the stimulus into the centre. These data were obtained in full dark adaptation, but similar curves were also obtained with partially dark-adapted and with light-adapted units.

The receptive field organization of the lone unit with a surround is shown in Fig. 4. This unit was an 'on-off' cell, with 'off' responses elicited by stimuli falling on its centre, and 'on' responses from stimuli in the surround (including annular stimuli). This cell was encountered after the preparation had been partially light-adapted, but its organization did not change in the hour during which it was being studied, with further changes in adaptation.

These experiments confirm our earlier findings, in which only large stimuli were used. The relationship between rhodopsin concentration and the logarithm of retinal sensitivity, whether determined by the ERG or the ganglion cell, is linear. This agrees with unpublished findings of Dowling and Ripps in the all-rod retina of the skate in which ganglion cell responses to small stimuli were compared with spectrophotometric data obtained by rapid scanning of the visible spectrum.

The discrepancy between the findings of our study and those of Brown and Rojas, regarding the prevalence of ganglion cell receptive fields with antagonistic surrounds, is disturbing, and not easily resolved. One possible explanation is that units without surrounds were preferentially selected because of the electrodes used, or because

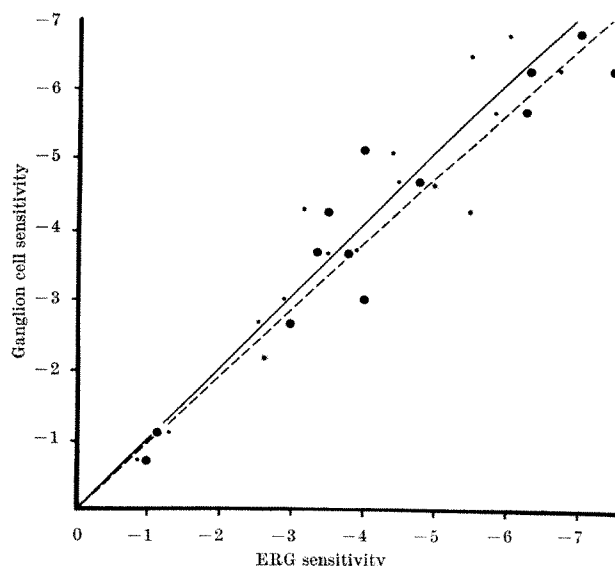


Fig. 3. A comparison between ganglion cell and ERG sensitivity: combined data from six experiments. Both large and small spots were used to determine ganglion cell sensitivity. Two lines providing a best fit for the large spot and the small spot data points are indicated. The solid line best fits the small spot data and the interrupted line best fits the large spot data. The difference in slopes between these two lines is probably negligible. *, Same data points for large and small spots.

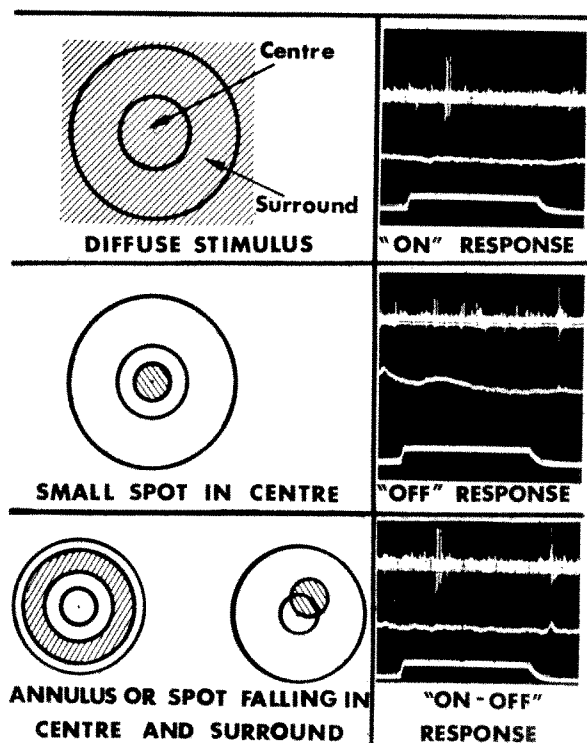


Fig. 4. Receptive field organization of a ganglion cell of the isolated rat retina. A single unit was found which showed centre surround organization. A diffuse stimulus produced an on response in this cell. A small spot presented in the region of the recording microelectrode produced an off response. An annular stimulus or a slightly eccentric spot produced an on-off response.

of the preparation. Another explanation might be that of sampling error. We know of no way to resolve the matter at the present time.

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Enzyme Flexibility in *Drosophila melanogaster*

POLYMORPHISMS for genetically determined enzyme variants have been reported in humans¹, *Drosophila*² and several other groups of organisms, but there is no adequate explanation for their occurrence. Genetic polymorphisms can be maintained in numerous ways³, but Haldane⁴ and Mayr⁵ have emphasized that geographical

or temporal changes in the environment could place a premium on metabolic flexibility and that selection for heterozygotes or alternating selection for different homozygotes might establish the necessary enzyme heterogeneity in the population.

Natural populations of *Drosophila melanogaster* are often polymorphic for two electrophoretic variants of alcohol dehydrogenase (*Adh*^F and *Adh*^S) each determined by a single genetic locus⁶, but in most laboratory populations investigated the *Adh*^F gene has been fixed. Our evidence shows that the various forms of alcohol dehydrogenase have different thermolabile properties and are differentially affected by the presence of ethanol in the culture medium, which suggests that variation in temperature, strains of yeasts and their conditions of growth should be considered as environmental factors that may be relevant to the maintenance of this polymorphism.

Homogenates of twenty third instar larvae in 1 ml. 0.05 M orthophosphate buffer (pH 7) of each of the three genotypes *Adh*^F/*Adh*^F, *Adh*^S/*Adh*^S and *Adh*^F/*Adh*^S on a common genetic background were centrifuged at 30,000g for 20 min. The supernatants were assayed for alcohol dehydrogenase activity in a Unicam 'SP 800' spectrophotometer by following the increase in absorbance at 340 mμ after addition of the enzyme extract to a solution containing nicotinamide adenine dinucleotide (NAD) and isopropanol. Total protein content of each extract assayed was determined by the Folin method as modified by Lowry *et al.*⁷.

Third instar larval extracts of *Adh*^F homozygotes have a higher enzyme specific activity than extracts from *Adh*^S homozygotes (Table 1). In assays at 25° C, enzyme activity in extracts of *Adh*^F/*Adh*^S heterozygotes is intermediate between the activities of the two parental extracts. Similar results have been described for extracts of adult flies⁸.

After the enzyme assays the larval extracts of the three genotypes were kept for 10 min in a 40° C water bath and the enzyme activity remaining was measured. Following this heat treatment, enzyme extracts from the *Adh*^S homozygote are more stable than the extracts from the *Adh*^F homozygote or from the *Adh*^F/*Adh*^S heterozygote (Table 1). Heat-treated extract from heterozygotes, however, has a higher specific activity than extracts from either of the two homozygotes.

Table 1
Mean specific activities*

<i>Adh</i> genotypes	Larval extract	s.d. \bar{z}	Heat-treated extract	s.d. \bar{z}	Loss of activity (per cent)	Culture medium with 6 per cent ethanol	s.d. \bar{z}	Increase in activity (per cent)
<i>Adh</i> ^F / <i>Adh</i> ^F	8.5	0.33	1.2	0.092	85.9	12.4	0.57	45.8
<i>Adh</i> ^S / <i>Adh</i> ^S	2.9	0.13	1.5	0.188	45.6	3.5	0.23	20.7
<i>Adh</i> ^F / <i>Adh</i> ^S	5.4	0.32	2.0	0.108	61.6	6.8	0.28	30.8

* Each value is the mean of eight separate assays. Enzyme assays of treated and untreated extract were run concurrently.

Homogenates of single third instar larvae homozygous for either *Adh*^F or *Adh*^S normally show three major bands and two minor bands after electrophoresis on polyacrylamide gels and subsequent tetrazolium staining. The major bands designated F₁, F₃ and F₅ in *Adh*^F/*Adh*^F (S₁, S₃ and S₅ in *Adh*^S/*Adh*^S) from the most anodal, are also present in *Adh*^F/*Adh*^S heterozygotes together with a further three bands which occupy positions in the gel intermediate between the corresponding parental bands. To test the effect of the heat treatment on the *Adh* phenotypes, electrophoresis on polyacrylamide gels was carried out on heat-treated extracts from each of the three genotypes. In extracts from *Adh*^F and *Adh*^S homozygotes the F₃ and S₃ bands respectively were absent and the F₁ and S₁ bands were very faint confirming previous work⁹. Two distinct bands were found in gels run with heat-treated heterozygote extract and these corresponded to bands F₃ and S₃. It has previously been shown^{10,11}

that the F_1 and F_3 bands represent enzyme bound to NAD and the heat stability in a gel of the F_3 moiety is enhanced after dialysis against NAD when the F_3 band is converted to F_2 and F_1 . Presumably the three bands in *Adh^S* homozygotes are also explicable in terms of NAD binding to the enzyme and bands S_1 and S_3 will be more heat stable than band S_2 as the bound NAD will stabilize the enzyme. It is not yet known whether the three extra bands found in heterozygotes also result from differing degrees of NAD binding to the enzyme; some of these bands may represent hybrid enzyme molecules. Nevertheless, the NAD bound enzyme present in heterozygotes appears to be more heat stable than the NAD free enzyme.

To determine whether the level of alcohol dehydrogenase in the three genotypes was affected by the food medium on which the *Drosophila* were cultured, eggs were collected and transferred to a dead yeast/glucose medium supplemented with 6 per cent ethanol. Samples of third instar larvae of the three genotypes cultured on this medium were assayed for alcohol dehydrogenase activity. With isopropanol as the assay substrate, there is a differential increase in enzyme activity in extracts of the three genotypes, *Adh^F* homozygotes having the largest increase (Table 1). It therefore appears that environmental changes produce differences in enzyme activities in different *Adh* genotypes. There is marked genotype environment interaction.

Experiments are being carried out to investigate whether the different levels of alcohol dehydrogenase activity in *Drosophila* have any adaptive significance. Preliminary results suggest that they have. Four bottle cultures were started with the alleles described at a gene frequency of 0.5 *Adh^F* and maintained at 25° C by mass transfer every 2 weeks. The standard dead yeast/glucose culture medium was supplemented with 6 per cent ethanol for two of the populations. After eighteen generations the gene frequency in the two control populations had not changed, whereas in the two ethanol supplemented cultures the gene frequencies of *Adh^F* had increased to 0.73 and 0.82.

In natural habitats yeasts are an important factor in the ecology of *Drosophila* species^{12,13} and there is probably considerable variation in the amounts of ethanol produced by yeast fermentation in *Drosophila* habitats. It is possible that the rate of ethanol oxidation in *Drosophila* is not directly related to the level of alcohol dehydrogenase activity. A polymorphism exists in human populations for an atypical human liver alcohol dehydrogenase, however, which is reported¹⁴ to produce a faster drop in blood alcohol concentration due to increased ethanol oxidation. *D. melanogaster* inhabits a wide range of environments and flies have been found in habitats with temperatures near 40° C. Thus the variation found in *Drosophila* alcohol dehydrogenase may be relevant to natural populations.

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Long Chain Fatty Acid Formation: Key Step in Myelination studied in Mutant Mice

MYELIN is the most stable membrane known and the fact that it contains ten times as much long chain sphingolipid as any other known membrane structure has led to the suggestion that the long chain fatty acids (>18 carbon atoms) in these sphingolipids are concerned in the stability of myelin^{1,2}. The finding^{3,4} of a myelin deficient mouse mutant whose galactolipids and sphingomyelin lack long chain fatty acids led us to investigate whether the elaboration of long chain sphingolipid molecules is a key step in myelin formation. The comparison of histological and biochemical events related to myelination in normal and myelin deficient mutant mice supports the hypothesis that in the events leading to adult myelin, the appearance of fatty acids with 24 carbon atoms follows the appearance of cerebroside and sulphatides in myelin.

The two myelin deficient mutant mice, "Quaking" and "Jimpy"⁴, manifest symptoms at the time of myelin formation, around the tenth day after birth. Although they have the same apparent phenotype, characterized by an abnormal gait, tremor and seizures, they have a different genotype; the Quaking mutation is autosomal and recessive; the Jimpy mutation is sex linked, X linked, implying that genes located on at least two chromosomes are involved in myelin metabolism and control. Unlike Quaking mice, Jimpy mutants do not reach adulthood; their disease is more severe and histological and biochemical data relate this to an earlier stage corresponding to myelination. Glial cells normally multiply after birth and reach maximum density just before the onset of myelination. Second, they extend processes along the axonal fibres, so forming a myelinating glia constituted of oligodendrocytes whose function is analogous to that of the Schwann cell in the peripheral nervous system. In the Jimpy mouse there is a lack of myelinating glia⁵; by contrast, the normal types of neuroglial cells are present in the Quaking mouse⁶, but the glial cells may be qualitatively deficient, for vacuoles and inclusions have been demonstrated inside their cytoplasm⁷.

The biochemistry of myelination is still rather obscure. Galactolipids are the only lipids characteristic of brain white matter⁸. Experiments with radioactive isotopes have shown that cerebroside and sulphatides are synthesized at birth⁹⁻¹¹; galactolipids, especially cerebroside, are only detectable in a significant amount at the onset of myelination¹² and possibly are of key importance in this process⁹: the other lipid components of myelin have been shown to occur in other membranes and are already present in large quantities at birth. In the Jimpy mouse, the cerebroside and sulphatide content is negligible¹³, which correlates with a very early impairment in the process of myelin formation. By contrast, cerebroside and sulphatides are formed in the Quaking mouse although they are deficient in long chain fatty acids with 24 carbon atoms.

Myelin was isolated from the Quaking mouse to study whether myelin was only deficient in quantity or whether its composition was abnormal in relation to a maturation process involving the appearance of long chain fatty acids in sphingolipid molecules. We compared Quaking adult myelin with that of apparently normal adult litter mates using the method of Norton *et al.*¹⁴. We also isolated by the same method myelin from 12 day old apparently normal mice. The proportions of proteolipid to lipid showed little variation between young and adult stages. The values obtained for the molar ratios of phospholipids, cholesterol and galactolipids were similar to those observed by Norton *et al.*¹⁴ and Eng *et al.*¹⁵ for the rat, with a molar ratio of galactolipids to phospholipids of 0.28 at 12 days and 0.64 at 3 months. Comparison of the fatty acid pattern in 12 day old myelin lipids with that of adult brain myelin lipids confirmed the increase with age of

stearic and oleic acids¹⁶; moreover, the proportion of long chain fatty acids (with 24 carbon atoms) was 10–11 per cent of the total fatty acids in adult normal myelin (Table 1). By contrast, the fatty acids of 12 day old myelin included only small amounts of long chain fatty acids (Table 1), although cerebrosides and sulphatides were already present. In the Quaking mouse, the lipid composition and protein content were closely related to the values observed in the early days of myelination, there being a great deficiency in long chain fatty acids (Table 1).

Table 1. FATTY ACID COMPOSITION (PER CENT) OF BRAIN MYELIN LIPIDS

	Adult normal	12 day old normal	"Quaking" adult
C ₁₄ :0*	0.30	1.40	0.30
C ₁₆ :0	12.64	27.43	24.82
C ₁₈ :0 + C ₁₈ :1	45.87	35.15	43.50
C ₂₀ :0 (?)	5.85	11.32	8.43
C ₂₀ :1	7.13	—	0.50
C ₂₂ :0	5.98	12.79	10.50
C ₂₂ :1	2.01	0.31	0.86
C ₂₄ :0	4.50	2.14	1.08
C ₂₄ :1	7.09	2.04	0.66
C ₂₄ :2	3.69	1.27	0.78

* Gas chromatography of fatty acid methyl esters of brain myelin lipids on 10 per cent SE 52 column. Temperature, 225°C. Flow rate, 25 ml. nitrogen/min. The results are the average of three analyses. The normal mice correspond to apparently normal homozygote and heterozygote littermates of Quaking mice.

* Fatty acids are identified by the number of carbon atoms and number of double bonds (for example C₁₈:0 = stearic acid); h indicates the presence of a 2-hydroxy-group.

It seems therefore that in the normal maturation process leading to adult myelin, the appearance of fatty acids with 24 carbon atoms follows the appearance of cerebrosides and sulphatides. A deficiency in long chain sphingolipid molecules as observed in the Quaking mouse may lead either to cessation of myelination or to the formation of unstable myelin² and may explain the loose configuration of early myelin¹⁷. The key step (blocked in the Quaking mutation) may be the formation of these long chain fatty acids or their association with a cerebroside precursor through the action of chain length specific enzymes. Myelin membrane proteins may also be concerned: they are known in other membranes to determine the association of lipids containing specific fatty acids¹⁸.

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Genetic Effects of ¹³¹I in Mice

We have already studied¹ the genetic effects of phosphorus-32 and warned against its indiscriminate use before or during the child bearing age. We report here an investigation of the genetic effects of ¹³¹I. ¹³¹I is a beta and gamma emitter with a half life of 8.04 days and an energy spectrum of 0.188 \bar{E}_B MeV. It is widely used for diagnostic and therapeutic purposes in doses ranging from 16 to 200 μ Ci and 50 to 700 μ Ci respectively in human medicine.

A survey of the medical history of ¹³¹I indicates that a dose of 563 μ Ci used to treat thyroid carcinoma induces sterility². In another report³, ¹³¹I injected into the pregnant woman was found to be concentrated in the 18 week old foetus and its immature Graafian follicles. Bengtsson *et al.*⁴ have also reported that ¹³¹I is concentrated in the Graafian fluid.

Male mice of inbred strain C3H/He aged 10–12 weeks were given an intraperitoneal injection of ¹³¹I in the form of sodium iodide in 0.5 ml. of isotonic saline solution in doses of 0.2, 0.5 and 0.7 μ Ci. It should be noted that intraperitoneal injection is not the same as the oral or intravenous routes used medically. 24 h after injection, each treated male was mated with three untreated females of the same age. Vaginal plugs were examined every morning and females which had copulated were replaced by fresh females. The mated females were dissected on the fifteenth day of gestation and the number of live and dead embryos (including deciduomata) were recorded. Control observations were made on females mated to untreated males.

Table 1 gives the results of mating during the first four weeks, and they show that injection of ¹³¹I had a demonstrable effect on the intrauterine deaths.

The observations clearly indicate mutagenic effects of ¹³¹I in mice. Fig. 1 shows graphically the effect during the different periods of mating (1–4 weeks).

The peak period for all doses seems to be the second and third weeks. The sperm screened during these periods would probably have been in the spermatid stage at the time of injection⁵. The high sensitivity of spermatids agrees with the observations of others using X-rays⁶ and ⁹⁰Sr (ref. 5). The significantly high incidence of intrauterine deaths during the first and the fourth weeks suggests that ¹³¹I could also cause chromosomal breaks in spermatozoa and spermatocytes. The results of this investigation point to the possible hazards of any indiscriminate use of radioiodine in human medicine.

The mechanism of mutagenic action needs further exploration.

Table 1. RESULTS OF MATINGS (1–4 WEEKS) OF MALES INJECTED WITH ¹³¹I (DATA FOR FERTILE MATINGS ONLY)

Dose	♂♂ treated	Weeks	Females dissected	Total No. of implants	Dead	Per cent dead	Dead per female
Control	50	1	98	615	22	3.58	0.22
		2	52	411	21	4.86	0.40
		3	67	483	11	2.27	0.16
		4	59	481	22	4.57	0.37
Total		1–4	276	1,990	76	3.81	0.27
0.2 μ Ci	100	1	57	435	49	11.2	0.85
		2	109	698	101	14.46	0.92
		3	112	743	62	8.3	0.55
		4	121	963	49	5.08	0.40
Total		1–4	399	2,839	261	9.19	0.65
0.5 μ Ci	100	1	60	498	44	8.83	0.73
		2	133	1,032	150	14.54	1.12
		3	104	843	104	12.33	1.00
		4	113	892	106	11.86	0.9
Total		1–4	410	3,265	404	12.37	0.98
0.7 μ Ci	100	1	87	909	132	14.5	1.5
		2	76	653	105	16.0	1.3
		3	105	960	128	13.3	1.2
		4	96	796	60	7.53	0.62
Total		1–4	364	3,318	425	12.8	1.16

P value for the comparison between control and 0.2 μ Ci is $0.02 \leq P \leq 0.05$ and for the comparison between 0.5 μ Ci and 0.7 μ Ci it is $0.50 \leq P \leq 0.70$ whereas for all other comparisons $P \leq 0.001$. (The conclusions are based on 1–4 week totals only.)

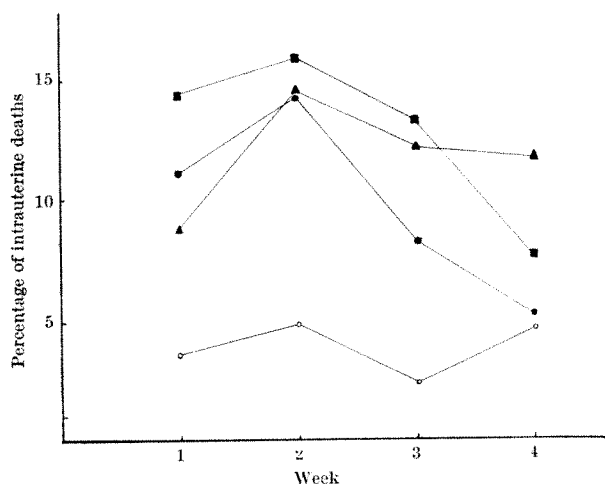


Fig. 1. The percentage of intrauterine deaths when matings took place 1-4 weeks after the males were injected with ^{131}I . ○, Control; ●, 0.2 μCi ; ▲, 0.5 μCi ; ■, 0.7 μCi .

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Antineoplastic Components of Marine Animals

REFERENCES to the biological properties of marine organisms date back to antiquity¹. For example, hieroglyphics on the tomb of the Egyptian Pharaoh Ti (approximately 2700 BC) described the poisonous puffer fish *Tetraodon stellatus*. Perhaps the earliest recorded use of a marine organism in primitive medical practice occurred when the Roman, Plinius Secundus (AD 29-79), recommended that the sting unit of the stingray be ground up and used for treatment of toothache and in obstetrics. The first modern pharmacological and chemical studies of a substance from a marine animal probably involved tetrodotoxin from the poisonous puffer fish (for leading references see refs. 2 and 3). In recent years, extracts from certain sponges and coelenterates have been shown to have antibiotic properties⁴⁻⁶. There have also been indications that marine invertebrates produce various other potentially medically useful components⁷⁻¹¹. Most important from the standpoint of cancer chemotherapy have been observations that starfish meal¹²⁻¹⁴ and the peanut worm *Bonellia fuliginosa*^{15,16} and that partially purified material from certain sea cucumbers [Echinodermata] have antitumour activity against sarcoma-180 and Krebs-2 ascites tumour^{10,17-19}. Also certain clam¹⁸ (Mollusca) extracts and material from oysters have antitumour properties⁹⁻¹¹.

In 1968 we reported²⁰ evidence for antineoplastic agents in terrestrial arthropods. During an analogous investigation of marine animals we have evaluated components from many marine invertebrates and vertebrates from a broad geographic area (Atlantic and

Pacific coasts of North and South America and the coasts of Asia). We assessed antineoplastic activity using the Walker 256 carcinoma (intramuscular) and both a lymphoid (L-1210) and lymphocytic leukaemia (PS) as developed at the National Cancer Institute.

We wish to report that at present a few representative species have given very encouraging results. Two marine animals from the Gulf of Mexico provide a general illustration. An aqueous 2-propanol extract of the bryozoan *Nugula nerita* in several doses led to 168-200 per cent life extension (PS system). Similarly an ethanol extract of *Molgula occidentalis* (Chordata/Tunicata) at 50 mg/kg gave 177 per cent life extension using the PS leukaemia. (To obtain the extract the marine animal was pretreated by extraction with ligroin and aqueous 2-propanol. Accordingly, the ethanol extract represents a partial concentration of active component(s).) In each case the material was given intraperitoneally (in carboxymethyl cellulose) on the day of transplantation of tumour into BDF₁ mice and continued for each of 10 days. Active components are now being isolated from these specimens and others, including (Bryozoa) *Amathia convoluta*, *Bugula nerita*, *Thalamaporella gothica floridana*; (Coelenterata) *Anthopleura xanthogrammica*, *Renilla mulleri*, *Tealis coriacea*; (Echinodermata) *Astropecten scoparius*, *Luidia clathrata*, *Lytechinus variegatus*, *Mellita quinqueperforata*, *Pisaster ochraceus*; (Mollusca) *Cristaria plicata spatiosa*, *Gryphus stearnsi*, *Haliotis ovina*, *Macrocallista nimbosa*, *Turbo (marmarostoma) stenogyris*; (Arthropoda) *Matuta lunaris*; (Chordata/Tunicata) *Clavelina picta*, *Molgula occidentalis*, *Styelis plicata*; (Chordata/Pisces) *Porichthys porossissimus*. One of these, the vertebrate *Clavelina picta*, has resulted in a life extension of 200 per cent (at 400 mg/kg using the PS method).

We were not surprised to confirm the antineoplastic activity of extracts of marine invertebrates, but to discover antineoplastic components among the marine vertebrates represents a stimulating new development. There are more than a million species of marine invertebrates and more than 25,000 known species of fishes, and so our results suggest that marine animals will be a particularly fruitful and interesting source of potentially useful antineoplastic agents.

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Conversion of Palmitate to Respiratory Carbon Dioxide by Foetal Tissue of Man and Monkey

ALTERATION in the amount of fat deposition in the foetus is clinically well documented for anencephalic, diabetic and low-birth weight infants¹⁻³ and indicates that the rates of synthesis and degradation of foetal lipids, like those of the adult, are influenced by environmental and hormonal factors. It also suggests that the human foetus anabolizes or catabolizes lipids at different rates in response to pathological conditions of gestation. Observations in man and rabbit have shown that the foetus anabolizes lipids essentially from carbohydrate precursors and does not apparently catabolize lipids^{4,5}. If this is true, fatty acids would not be catabolized to respiratory carbon dioxide (CO₂) by foetal tissue in normal conditions. The assumption is open to question because slices of human foetal liver incubated with labelled fat have been shown to metabolize this substrate to respiratory carbon dioxide⁷.

We attempted to show that the foetus is able to degrade fatty acids *in vitro*. Slices of placenta, brain, liver and lung from human and monkey foetuses obtained at various periods of gestation by interruption of normal gestation were studied. Each incubating vessel contained 2.2 mmoles of purified palmitate-1-¹⁴C (specific activity 15 μ Ci/mmol obtained from New England Nuclear Corporation) dissolved in a total volume of 3 ml. of albumin-Krebs-Ringer phosphate pH 7.4 with 10 mmoles of added glucose⁶. The purity of radioactive palmitate, determined by gas chromatography, was found to be 98 per cent.

Brain, liver and lungs were incubated for 1 h in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. The CO₂ was trapped in alkali, placed in the centre well of the incubation flask (0.2 ml. of a carbon dioxide-free 5 per cent solution of sodium hydroxide). At the end of the incubation this solution was washed into centrifuge tubes containing water, the CO₂ was precipitated as BaCO₃ and transferred quantitatively to scintillation vials containing a mixture of toluene with 4 per cent 'Cab-o-Sil' and 4 g/l. of BBOT (Packard Instruments)⁷. The radioactivity was determined in a scintillation counter (counting efficiency: 82 per cent).

Because of the dilution of the radioactive palmitate by the amount of unlabelled fatty acids in the incubating system⁸, the incorporation of palmitate to CO₂ was expected to be low. As control, slices of placental tissue were incubated in a nitrogen atmosphere or at 0°C with labelled palmitate. No significant radioactivity could be detected in the trapped carbon dioxide. Each sample was counted long enough to obtain a standard deviation of ± 3 per cent (95 per cent confidence limits). All data were submitted to the Student's *t* test or analysis of the variance.

Fig. 1 and Table 1 show that palmitate is oxidized to CO₂ by each foetal, newborn, and adult tissue studied. The degradation of palmitate to CO₂ by human and monkey placental tissue increases as a function of time ($P < 0.01$ at 60 min). Liver and lung tissue of both man and monkey catabolizes palmitate to CO₂ more actively than brain tissue ($P < 0.01$). Though the problem of

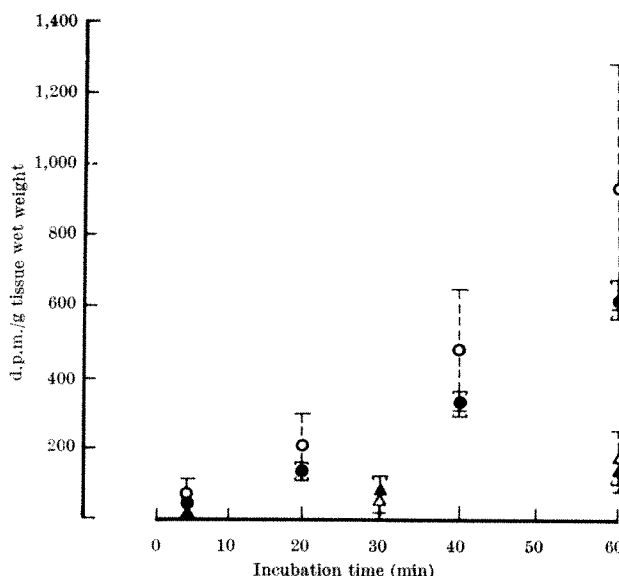


Fig. 1. Respiratory ¹⁴CO₂ production from palmitate-1-¹⁴C by placental tissue. The results are the mean \pm s.e. of four 13-14 week (○), and six term (●) human placentas. The results of two 60-90 day (△) and term (▲) monkey placentas are also shown. All determinations were carried out in duplicate.

dilution in tissue by endogenous palmitate is difficult to resolve, the findings suggest that palmitate is either selectively retained as brain lipid, diluted into a large cellular free fatty acid pool, or does not cross into brain cells as readily as in other tissue. The first hypothesis is the most probable because the net synthesis of lipids from palmitate in the human and monkey foetal brain tissue is as fast as that of liver, lung, or placenta⁸. In monkey tissue and in the human placenta, the rate of production of respiratory CO₂ from palmitate does not vary with development. Adult and newborn lung tissue, however, catabolizes palmitate to CO₂ more actively than foetal lung tissue. This could be the result of an increased rate of palmitate catabolism by lung tissue after delivery. At birth this tissue assumes its respiratory function, so it is to be expected that the overall metabolism of lung lipids will increase^{9,10}, resulting in increased CO₂ production from glucose or fatty acid precursors.

The oxidation of palmitate to CO₂ by these tissues demonstrates the presence in the foetus of the β , α or ω oxidative enzymes which catabolize the stepwise cleavage of two carbon units from the long chain fatty acyl-CoA beginning from the carbonyl end¹¹. It sheds some doubt on the consensus of opinion that the foetus only anabolizes lipids⁴, but suggests rather than metabolic pathways are available to oxidize fatty acids in foetal tissue, as they are in skeletal muscle, liver, heart and kidney of the adult animal¹².

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Table 1. *In vitro* OXIDATION OF PALMITATE-1-¹⁴C TO RESPIRATORY ¹⁴CO₂ (d.p.m./g tissue wet weight)

Tissue	Gestational age	Brain	Lung	Liver
Human n=4	12-13 weeks	156 \pm 49	278 \pm 151	977 \pm 115
Monkey n=2	60 days	47, 27	75, 49	195
	90 days	26, 29	120, 109	177, 237
	120 days	44, 26	112, 86	169, 99
	159 days	39, 57	88, 62	133, 211
Average n=8		*36.7 \pm 4.1	87.6 \pm 8.8	174.4 \pm 17.6
Newborn monkey n=2	1 day old	44, 52	237, 286	213, 161
Adult monkey n=2		44, 44	312, 377	91, 130
Average n=4		46.0 \pm 2.0	†303.0 \pm 30	148.7 \pm 25.7

The results are expressed as mean \pm s.e.m. or individually.

n, Number of experiments carried out in duplicate determinations.

* Different from foetal lung or liver mean values ($P < 0.01$).

† $P < 0.01$ when compared with foetal lung.

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Failure of 5 α -Dihydrotestosterone to initiate Sexual Behaviour in the Castrated Male Rat

In most mammalian species the principal steroid product of the testes is testosterone, 17 α -hydroxyandrost-4-en-3-one. In addition to being one of the most potent naturally occurring androgens it has been considered to be the active form of the hormone at target tissue level. Recent reports suggest, however, that a metabolite of testosterone, 17 α -hydroxy-5-androstan-3-one (5 α -dihydrotestosterone (5 α -DHT)), may be the active form of the hormone^{1,2}. Several target organs (prostate, seminal vesicle and penis) rapidly convert testosterone to 5 α -DHT (refs. 1 and 2). This conversion occurs before the attachment of the hormone to the receptors³ and seems to be essential for subsequent protein synthesis. Additional evidence for this function of 5 α -DHT comes from studies on patients with testicular feminization; tissue biopsies from the genital organs of such patients cannot convert testosterone to 5KDNT (ref. 4). Testosterone can also activate both male and female sexual behaviour and is more potent than oestrogens in inducing libido in women⁵. Recently the enzyme system needed to convert testosterone to 5 α -DHT has been demonstrated in the hypothalamus of the rat and dog^{6,7}, which suggests that 5 α -DHT may also be the active form of testosterone in inducing sexual behaviour. In the ovariectomized female rabbit, however, 5 α -DHT is devoid of any oestrus-inducing properties at doses from 2–20 mg per day⁸. 5 α -DHT cannot be converted to oestrogenic metabolites *in vivo*⁹, which suggests that the aromatization of androgens may be of significance in the activation of sexual behaviour. This preliminary report concerns the failure of 5-DHT to initiate sexual behaviour in the castrated male rat.

Three groups (six animals each) of sexually inexperienced mature male Wistar/Sprague-Dawley rats were housed in community cages with 14 h light and 10 h dark per day. Food and water were provided *ad libitum*. Animals in group 1 served as intact controls. Six weeks before testing began, all the rats in groups 2 and 3 were bilaterally castrated. Testing was carried out once daily during the second half of the normal light cycle (1400 to

1700 h) in large open-fronted cages. In each test the male was placed in the cage 2–3 min before the female and was removed from the cage after the female. Observations were made either for 15 min or until the occurrence of the first intromission following ejaculation. If no activity took place in the first 7.5 min the test female was replaced. Ovariectomized females were brought into oestrus with a single subcutaneous injection of 100 μ g oestradiol benzoate 48 h before testing. After fourteen daily tests, rats in group 2 received daily subcutaneous injections of 100 μ g testosterone propionate (TP) for 8 days and animals in group 3 received 125 μ g 5 α -dihydrotestosterone propionate (5 α -DHTP) daily for 8 days. Testing continued for a further 9 days. The rats were autopsied after the final test and the body weights and seminal vesicle and prostate weights were recorded.

During the first week of testing none of the animals showed any sexual activity (Table 1). A high level of performance was reached by the intact controls and TP-treated animals by the final week, whereas only one rat treated with 5 α -DHTP responded; this consisted of a single mount in one test (Table 1). During the first week of treatment the performance of the TP males was lower than that of the intact group, but by the final week the activity of the two groups did not differ significantly, except for ejaculation latency which was significantly longer in the TP treated males ($P < 0.05$). The behavioural responses show that the period of treatment with TP was sufficient and suggests that the failure of 5 α -DHTP to activate sexual behaviour was probably not the consequence of the length of the injection period. The autopsy weights (Table 2) confirm that 5 α -DHTP is almost as potent as TP and that complete restoration of the sex accessory glands is not necessary for the re-initiation of sexual behaviour.

Table 1. COMPARISON OF THE NUMBER OF RATS RESPONDING AND THE NUMBER OF POSITIVE TESTS OBTAINED IN INTACT CONTROL AND CASTRATED ANDROGEN-TREATED RATS

Period	Group No.	No. of rats	No. of tests	No. of rats responding	No. of positive tests
Control (weeks 1 and 2)	1	6	14	3	9
	2	6	14	1	1
	3	6	14	0	0
Experimental (week 3)	1	6	5	5	5
	2	6	5	4	3
	3	6	5	1	1
	1	6	4	5	4
	2	6	4	5	4
	3	6	4	0	0

Group 1, intact controls; group 2, castrated + 100 μ g/TP/day for 8 days; group 3, castrated + 125 μ g 5 α -DHTP/day for 8 days. Treatment began at the start of week 3.

Table 2. AUTOPSY WEIGHTS FROM INTACT CONTROL AND CASTRATED ANDROGEN-TREATED RATS (MEANS \pm S.E.)

Group No.	Treatment	Body weight (g)	Seminal vesicle weight (mg/100 g body wt.)	Prostate weight (mg/100 g body wt.)
1	Control	404.5 \pm 18.9	318.97 \pm 24.4	137.23 \pm 11.8
2	100 μ g TP	455.8 \pm 37.1	202.97* \pm 20.1	45.53 \pm 2.9
3	125 μ g 5-DHTP	455.7 \pm 38.3	113.68* \pm 11.5	44.03 \pm 5.8

* Significantly different from controls and from each other at 1 per cent.

† Significantly different from controls at 1 per cent.

Testosterone can be aromatized *in vivo* to give rise to oestrogenic metabolites¹⁰, and this has been suggested as a possible mechanism by which testosterone could induce sexual behaviour⁵. The failure of 5 α -DHTP to induce sexual behaviour in castrated male rats and ovariectomized female rabbits may therefore be because this compound cannot be aromatized⁹.

If this is the case, the aromatization of testosterone may be an important step in the activation of sexual behaviour in both the male and female. In support of this, androst-sterone—another androgen which cannot be metabolized to oestrogenic compounds—does not activate sexual behaviour in the ovariectomized rabbit (unpublished observations of C. B. and N. Vidal). Clearly, additional compounds will have to be tested before this concept can be finally accepted.

The 5-dihydrotestosterone propionate used in this study was kindly supplied by Dr P. Crabbe, Syntex de Mexico.

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Aglycogeusia: the Inability to recognize Sweetness and its Possible Molecular Basis

Two patients have been found who could not recognize the taste of any sweet substances. This has afforded us apparently the first opportunity to study this phenomenon; we have called it aglycogeusia.

The patients, both male, were aged 18 and 19, and had congenital idiopathic hypoparathyroidism. This diagnosis was made soon after birth in each patient following spontaneous seizure activity. Lower than normal serum concentrations of calcium were found in each and responded to treatment with exogenous calcium and/or vitamin D with the elimination of seizure activity. No specific aetiology of their hypoparathyroidism was established.

Detection and recognition thresholds for each of four taste qualities were determined in these patients by a slight modification of a forced choice, three stimulus drop technique previously described in detail^{1,2}. Sodium chloride was used for the salty taste, sucrose for sweet, hydrochloric acid for sour and urea for the bitter taste. In addition, their subjective taste responses to drops of saturated solutions of D-fructose, D-glucose, D-galactose, D-xylose, sucrose and phenylthiocarbamide (PTC) were determined. Responses to 1 per cent, 50 per cent and saturated solutions of sodium cyclamate were also obtained. These responses were determined on at least five occasions for each substance.

Detection thresholds (the lowest concentration of solute distinguishable from water) for each of the four taste qualities tested were within normal limits in each patient. Recognition thresholds (the lowest concentration of solute recognized appropriately) for three qualities, salt, sour and bitter, were also within normal limits. Neither patient, however, could usually recognize dilute and saturated solutions of sucrose as sweet. Oral administrations of other dilute and saturated sugar solutions were also not usually recognized as sweet. The subjective responses of the patients to these various solutions were similar and are shown in Table 1. Fructose and sucrose usually elicited a sour response, while glucose, galactose and 1 per cent sodium cyclamate usually elicited a bitter

response. D-Xylose, the only pentose in the group, usually elicited a salty response.

Taste acuity was studied in both patients over a period of two years, and on no occasion did they give a consistently sweet response to any sugar. Neither withdrawal of their replacement therapy, which lowered their serum calcium concentration, nor its reinstitution, which returned serum calcium concentration to normal, was associated with any change in their aglycogeusia. These observations suggest that the aglycogeusia in these patients is not associated with their abnormality of calcium metabolism, but rather is a genetic concomitant of their hypoparathyroidism, a disease which is often familial and may be transmitted as a recessive autosomal genetic defect³. Normal taste responsiveness to sugars has been observed in patients who exhibit hypocalcaemia after surgically produced hypoparathyroidism and in others with idiopathic hypoparathyroidism which occurs after the first several years of life. These normal taste responses which occur in presumably non-genetic abnormalities of the parathyroid glands contrast with the aglycogeusia found associated with a presumed genetic abnormality of the parathyroids.

Sensory, metabolic and other abnormalities have been associated with congenital idiopathic hypoparathyroidism (such as adrenal cortical insufficiency⁴, deafness (unpublished observation of R. I. H.) and moniliasis⁵). Both patients had bilateral sensorineural hearing losses, and one had severe cutaneous moniliasis. Aglycogeusia is probably another of the sensory defects found in these patients, and present, presumably, from birth.

Aglycogeusia in these patients is not associated with the inability to recognize PTC as bitter, for both recognized this substance as bitter even in dilute solutions. In addition, they recognized urea and sodium cyclamate as bitter. The distribution of aglycogeusia in the population at large is not known, for its presence has not previously been reported. It seems, however, to be a rare phenomenon, because in more than 2,000 patients with various acute and chronic illnesses in whom taste thresholds have been measured, it has appeared only in these two. Indeed, taste thresholds measured in one further patient with congenital idiopathic hypoparathyroidism were completely within normal limits.

Recently, a molecular theory of the taste of sweetness was proposed which related hydrogen bonding and specific molecular spatial parameters to specific degrees of sweetness^{6,7}. In this theory sweetness was related to the interaction between a sweet tastant and a receptor site, both of which possessed a "hard-acid" function AH and a "hard-base" function B (ref. 8). The sweet taste presumably occurs after the formation of a simultaneous intermolecular hydrogen bond between these complementary moieties. The optimum distance range between AH and B on both receptor and tastant in order for this interaction to occur is hypothesized to be about 3 Å.

Kubota and Kubo⁸ have recently postulated that one of the chemical features common to compounds which have a bitter taste, presuming an AH, B type receptor site, is that the presumed AH, B distance parameter is 1.5 Å and, as a result, a strong intramolecular hydrogen-bond is formed in the bitter compound.

Given these hypotheses, a possible explanation for the responses of our patients to galactose and glucose may be

Table 1. SUBJECTIVE TASTE RESPONSES TO VARIOUS SUBSTANCES IN TWO PATIENTS WITH AGLYCOGEUSIA

Substance*	Taste response
D-fructose	sour
D-glucose	bitter
D-galactose	bitter
D-xylose	salty
sucrose	sour
sodium cyclamate, 1 per cent	bitter
sodium cyclamate, 50 per cent	bitter
sodium cyclamate	bitter
phenylthiocarbamide	bitter

* Saturated solutions presented, except 1 per cent and 50 per cent sodium cyclamate.

based on stereochemical phenomena which exist in the intrinsic structure of the sweet tastants, the sugars themselves. Galactose and glucose have a stereostructure which makes possible the formation of intramolecular hydrogen bonds. For galactose in the favoured chair conformation in the axial hydroxyl group at C4 is sterically disposed to hydrogen bond to the ring oxygen atom and this may relate to its bitter taste to an aglycogeusia. Glucose as the β -D-anomer, with its hydroxyl constituents all in the unfavoured axial disposition, has its hydroxy-methylene group sterically disposed to hydrogen bond with the anomeric hydroxyl group, and this may account for its bitter taste. The only difference between xylose and glucose is the absence of the hydroxy-methylene group on the fifth carbon atom, and the patients called this taste salty. In patients with aglycogeusia, fructose elicited a sour response, possibly because of the relatively high acidic character of its anomeric hydroxyl group.

Our data suggest that taste specificity may be explained by a two-site receptor in which the molecular distance between the sites is of critical importance. The molecular abnormality in patients with aglycogeusia may be an abnormality in the intersite distance parameter.

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Specificity of Internal Signals in producing Satiety for Taste Stimuli

A GUSTATIVE sweet stimulus gives rise to a pleasant perception in fasting human subjects. The same stimulus will seem unpleasant after ingestion of a hypertonic glucose load^{1,2}. What is the nature of the internal signal responsible for this shift in perception from pleasant to unpleasant? McCleary has shown a relationship between the osmolality of the administered fluids and food intake and has suggested³ that satiety may be a result of extracellular dehydration. Other workers have also supported the importance of osmotic factors in alimentary behaviour and the close relationship between food and water intake⁴⁻⁷. We therefore decided to look at the influence of osmotic pressure in the affective transformation of taste perception in the human after ingestion of glucose and saline.

Experiments were conducted during the morning on twenty healthy, naïve, non-obese, fasted subjects of both sexes (no difference in the response was observed between sexes), ranging from 18 to 26 years old. They were offered two series of gustatory stimuli: NaCl at concentrations of 10, 5, 2.5, 1.25, 0.5 and 0.25 g/100 ml. in a volume of 25 ml. The sample was retained in the mouth for 15 s, was then expectorated and the subject gave a

quantitative response ranging from -2 to +2 describing the pleasantness or the unpleasantness of the taste. The scale was infinite, but the following grades were assigned: -2 very unpleasant, -1 unpleasant, 0 neutral, +1 pleasant, +2 very pleasant. After each stimulus the subject rinsed his mouth thoroughly with tap water. When all the test samples had been offered at random with double blind precautions and the responses had been recorded, the subjects ingested 200 ml. of a solution containing either glucose or sodium chloride. Ten subjects drank 50 g of glucose in solution while the other ten subjects received 15 g NaCl in solution through gastric tubing. All subjects therefore received an equal volume of a very hypertonic fluid. One hour later the same samples were offered, using the same method.

Fig. 1 shows that sweet perception, pleasant to fasted subjects, turned unpleasant at all concentrations after glucose ingestion (χ^2 test $P < 0.10$, Kruskal-Wallis test $P < 0.01$), but was not significantly changed after gastric

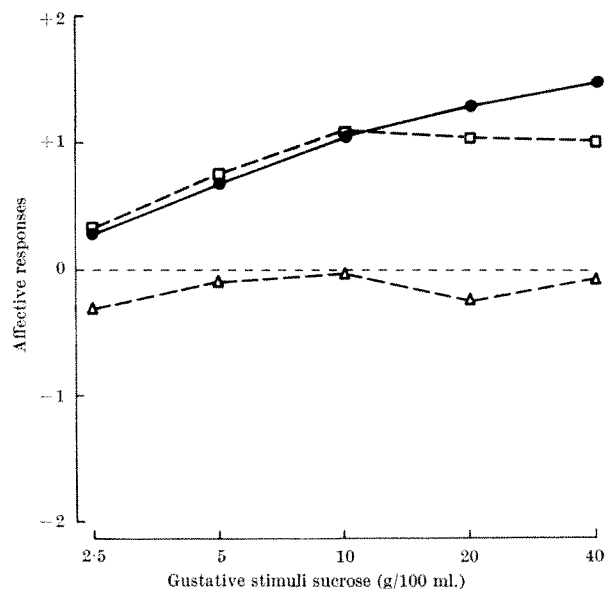


Fig. 1. Affective responses to varying concentrations of sweetness. ●—●, Control (N=20); △---△, 1 h after glucose load (N=10); □---□, 1 h after NaCl load (N=10).

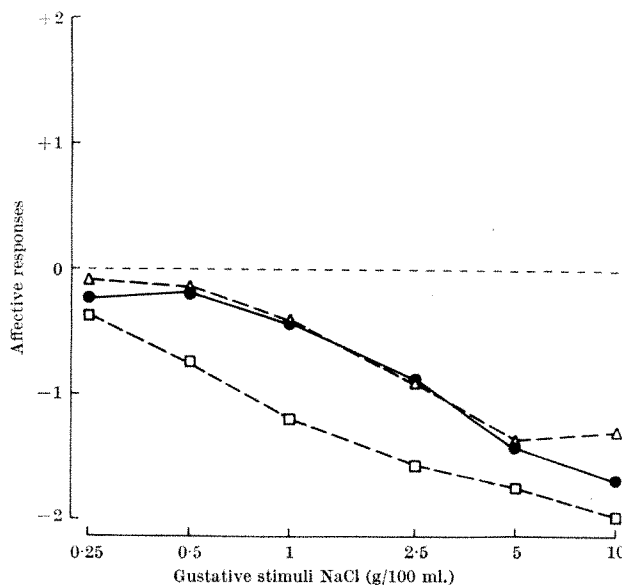


Fig. 2. Affective responses to varying concentrations of saline. ●—●, Control (N=20); △---△, 1 h after glucose load (N=10); □---□, 1 h after NaCl load (N=10).

hypertonic NaCl. There was a significant decrease only for the most hypertonic sucrose gustative stimulus (χ^2 test $0.2 P < 0.5$), but to a much smaller extent than after glucose ingestion. Fig. 2 shows the average responses after NaCl gustative stimuli on fasted subjects; the taste was unpleasant at all concentrations. Ingestion of the amount of hypertonic glucose needed to render unpleasant sweet stimuli did not significantly change the affective responses to NaCl, but these responses were significantly lowered, that is, slightly more unpleasant, after intragastric NaCl (χ^2 test $P < 0.01$) for all concentrations offered.

These results show that the shift from pleasant to unpleasant sweet perception for sucrose in man is probably not related to osmotic internal signals but seems to be more specific and related to the chemical nature of the ingested solute. They also show that internal signals are involved in affecting perception.

These results confirm the previous finding in rats which showed the importance of the gustative inputs in producing satiety^{5,8}. Arguments have been presented against⁸⁻¹⁰ or in favour of^{5,11,12} a specific satiety for sucrose and NaCl. Our results favour the second hypothesis.

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Decline of Tissue Noradrenaline under the Influence of a Mitotic Inhibitor

MATERIALS are transported down axons by two processes: a slow process of bulk axoplasmic flow moving at 1–6 mm/day and a fast transport process which is thought to be mediated by neurotubules and moves at 10^2 – 10^3 mm/day¹. Drugs such as colchicine, which inhibit mitosis by interfering with the formation of the mitotic spindle, interact also with neurotubules, indicating that the neurotubule and the mitotic spindle may be homologous structures². Colchicine inhibits both fast³ and slow⁴ intra-axonal protein transport, suggesting that neurotubules may be associated with both processes. Granules containing noradrenaline are transported down sympathetic nerves by a fast process⁵ which is inhibited by local application of colchicine⁶. Vinblastine, a mitotic inhibitor used in the treatment of certain cancers, affects formation of neurotubules⁷, and we have injected vinblastine intravenously into rats in an attempt to interfere with the intra-axonal transport of noradrenaline in sympathetic nerves generally.

Figs. 1 and 2 show the effects of a single dose of vinblastine on the noradrenaline content of heart, brain, spleen and vas deferens of male Sprague-Dawley rats (200–250 g). Noradrenaline was measured by a modification of the phenylethanolamine-N-methyltransferase

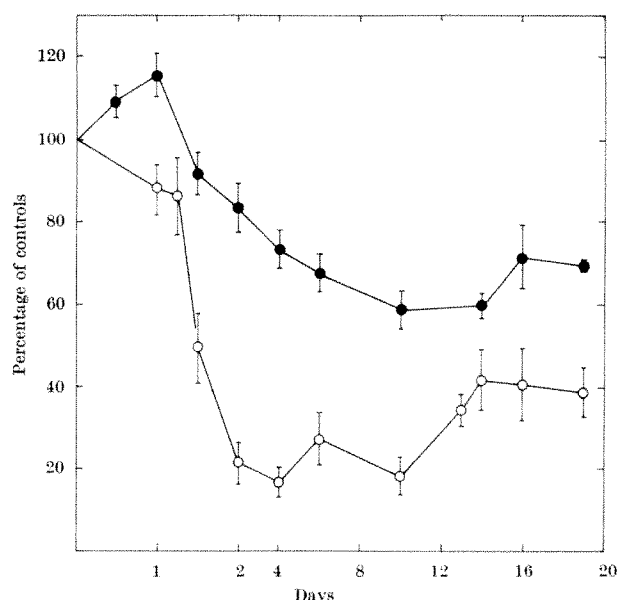


Fig. 1. The effect of a single dose of vinblastine (3 mg/kg, intravenously) on the noradrenaline content of rat heart (○) and vas deferens (●) expressed as a percentage of control values. Noradrenaline levels in control animals were: heart, $0.76 \pm 0.03 \mu\text{g/g}$; vas deferens, $11.26 \pm 0.60 \mu\text{g/g}$. Each point the mean (\pm s.e.) of not fewer than four and not more than eleven animals.

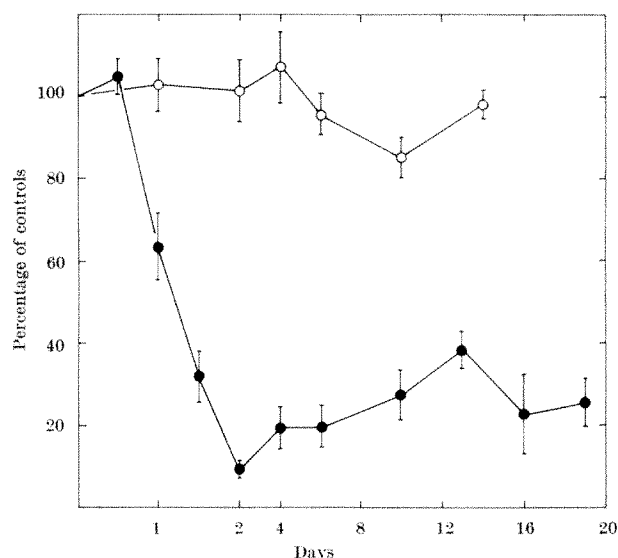


Fig. 2. The effect of a single dose of vinblastine (3 mg/kg, intravenously) on the noradrenaline content of rat brain stem (○) and spleen (●) expressed as a percentage of control values. Noradrenaline levels in control animals were: brain stem, $0.55 \pm 0.02 \mu\text{g/g}$; spleen, $1.32 \pm 0.10 \mu\text{g/whole spleen}$. Each point the mean (\pm s.e.) of not fewer than four and not more than eleven animals.

method⁸. Vinblastine reduced the noradrenaline contents of the spleen, heart and vas deferens to respectively 10, 20 and 50 per cent of control values, but had no effect on brain noradrenaline levels. There was a delay before the effect of vinblastine was seen: approximately 30 h in the heart and vas deferens and more than 12 h in the spleen. The rate of fall of noradrenaline levels in the vas deferens was much slower than that in the heart or spleen, possibly a reflexion of the slower turnover rate of noradrenaline in the former. In each case noradrenaline levels started to recover at about 10 days after administration of vinblastine; by 21 days cardiac noradrenaline levels had risen to 40 per cent of control values. Treated rats are being examined over longer time periods to determine whether complete recovery occurs.

The difference between the basal levels in heart and vas deferens (Fig. 1) suggests that because, unlike the heart, the vas deferens contains cell bodies as well as nerve endings, vinblastine could have blocked axonal transport so that the decline of noradrenaline levels could represent a decline of nerve-ending noradrenaline while the residual noradrenaline in the vas deferens could represent noradrenaline remaining in the cell bodies and axons. The rate of depletion of cardiac noradrenaline after vinblastine is, however, more rapid than that which follows inhibition of noradrenaline synthesis⁹ and so interference with axonal transport of noradrenaline cannot be the sole cause of the decline of noradrenaline levels. Possibly vinblastine interferes with the transport of some nutrient which is essential for noradrenaline storage at the nerve-ending and the delay period represents the time taken for existing stores of this nutrient to become exhausted. Despite its profound effect on sympathetic nerves, vinblastine did not cause any obvious impairment of function in motor nerves of treated rats, although vincristine, a related alkaloid, has been reported to cause degenerative changes in motor and sensory nerves in both rat¹⁰ and man¹¹.

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Tetrahydrocannabinol Isomers and Homologues: Contrasted Effects of Smoking

Two isomers of tetrahydrocannabinol (THC) are found in natural marijuana preparations. 99 per cent is in the form of $\Delta^1(9)$ -THC, which is very active when taken orally or smoked^{1,2}. A Δ^3 -THC homologue, synhexyl (pyrahexyl), has been found to be about one-third as active as Δ^1 -THC, given orally to the same individuals³. The study I report here compared measured doses of Δ^1 -THC, the Δ^3 -homologue (synhexyl), and Δ^3 -THC smoked by the same individuals, using a placebo and blind controls.

Ordinary commercial cigarettes were treated to give sets of four containing: (a) Δ^1 -THC, 12 mg, obtained from extraction of natural marijuana. The method for extraction has been described elsewhere³; the extract includes undetermined amounts of cannabinol and cannabidiol, as well as the specific amounts of Δ^1 -THC. (b) Synhexyl, 15 mg. (c) Δ^3 -THC, 15 mg. Both synhexyl and Δ^3 -THC were synthetic and measured gravimetrically. The synhexyl was provided by Dr R. Gwinn of Abbott Laboratories, N. Chicago, Illinois, and the Δ^3 -THC by Professor R. Mechoulam, School of Pharmacy, Hebrew University of Jerusalem. (d) Placebo, containing marijuana extract

from which all cannabinoids had been removed by prior extraction. Cigarettes were treated by injecting alcoholic extracts of each material throughout their length, with the exception of a small butt area, and evaporating to dryness under nitrogen. Cigarettes were stored until use in sealed tubes kept in a refrigerator. The tubes were identified only by code number, the order of treated cigarettes having been randomized.

Six experienced cigarette smokers were chosen as subjects on the basis of their habituation to nicotine and their ability to inhale fully and deeply. Each cigarette was smoked almost completely, using an unfiltered holder so that only a half-inch butt was left. Each of the four trials was separated by an interval of at least 48 h. The assignment of cigarettes was not known to the smoker or his monitor. Smoking involved deep inhalation and retention of the smoke for at least several seconds after each intake, the whole process being completed in 3–4 min.

The monitor obtained subjective reports of the effects of each smoking trial by frequent questioning during the entire span of action of the smoke. Pulse rate (three records) and the appearance of the conjunctiva were noted before each trial and at intervals of 10 min during trials.

Each of the six subjects had unmistakable effects from smoking the cigarettes containing 12 mg of Δ^1 -THC. Symptoms usually became apparent after three deep inhalations, and always by the time the cigarette was finished. Initial symptoms of numbness and tingling of the extremities, light-headedness, "floating" feelings and loss of concentration were followed by palpitation, sweating, tremulousness and weakness which lasted for varying times. Increasing mental impairment—described as difficulty in paying attention, difficulty in expression, mental confusion, loss of time sense, or a feeling of euphoria—was evident within 30 min. Some subjects complained of a dry mouth or burning feeling in the throat. Two subjects became quite sleepy and a third slept for about 2 h. Five of the six felt mentally clear between 60 and 90 min after smoking, but the one who slept was impaired for 4 h. Five of the six subjects had pulse rate increases of 10 or more beats/min, the mean increase being 20 beats. Five of the six subjects had unmistakable reddening of the conjunctiva, which appeared within minutes and tended to last as long as clinical effects were noticed.

Each of the subjects experienced definite effects from smoking cigarettes containing Δ^3 -THC, but these were less severe. Initial symptoms were light-headedness, numbness and tingling in extremities or face, fatigue and cold perspiration. Effects became evident before smoking was completed in four of the six subjects. Fatigue, drowsiness, sleepiness or a feeling of relaxation was prominent after 10 min and one subject fell asleep for 30 min. Impaired thinking and time sense distortion were not as prominent as with the Δ^1 -THC cigarettes. Subjects felt mentally clear 45 to 80 min after smoking. Only three subjects had a rise in pulse rate over 10 beats/min, the mean rise being 10 beats. Only three of the six subjects had reddened conjunctivae.

Compared with the other cigarettes, those treated with synhexyl elicited complaints of dry mouth or burning throat more often. Other symptoms were similar: light-headedness, numbness and tingling of face and extremities, drowsiness, lethargy and sleepiness, sweating and pallor, tremulousness, difficulty in thinking and speaking, mild confusion, and mild euphoria. As with the other active cigarettes, symptoms came on rapidly, but in one subject nothing more was noted than burning in the throat. The other five subjects had symptoms lasting from 45 to 150 min after smoking. Four of the subjects had increases in pulse rate greater than 10 beats/min, the average being 13 beats. Three of the six showed conjunctival injection.

The mere technique of smoking the placebo cigarettes, with deep inhalation and prolonged retention of smoke in the lungs, produced at least some symptoms in each

subject. Usually these were brief, no subject reporting any symptoms after 30 min. Early feelings of dizziness and light-headedness were the most common; one subject reported tingling of hands and nausea. Only one subject experienced more symptoms from the placebo cigarette than from the active ones, this being the subject who reported little effect with the Δ^3 -THC cigarette. Two subjects had pulse rate increases of more than 10 beats/min, the average being 8 beats. One had questionably reddened conjunctivae.

The effects of smoking Δ^1 -THC from marijuana extract were strongest and resembled those obtained from oral doses of the same extract in other experiments⁴. Onset was more rapid, and the experience briefer and more intense, when doses were smoked. Both the Δ^3 -THC isomer and its homologue, synhexyl, had activity like that of marijuana when smoked, but were considerably less potent than the Δ^1 -THC isomer. An estimate of the potency ratio might be 1:3 to 1:6. In the case of Δ^3 -THC, our results differ from those of others, who reported no effects from smoking almost twice the dose (400 μ g/kg) of the same isomer¹. Some unusual effects were noted from the placebo, but were not easily confused with those from the sizable doses of marijuana-like materials used in the present study. It is likely that the effects produced by placebo cigarettes were caused by the rather different technique for smoking marijuana, as compared with conventional, cigarettes.

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Inhibition by Drugs of Smoking Behaviour in Monkeys

THERE have been several analyses of the role of nicotine in smoking. Johnston¹ found that heavy smokers reported a pleasurable sensation when given nicotine, whereas non-smokers reported an unpleasant effect. Lucchesi *et al.*² showed that intravenous administration of nicotine diminished the number of cigarettes smoked by heavy smokers. Jarvik *et al.*³ recently obtained a similar result with nicotine taken orally. If, as these studies suggest, nicotine is an incentive in smoking, then drugs which block the action(s) of nicotine should influence smoking.

Because of the relative ease of controlling environmental contingencies, an animal paradigm of smoking behaviour has been developed in this laboratory⁴⁻⁶. In an attempt to modify smoking behaviour, various drugs have been given to four mature rhesus monkeys trained to puff cigarette smoke.

Puffing behaviour was initially instilled by making the monkeys suck on a tube in order to drink. The mouthpiece of a cigarette smoking apparatus⁵ was then substituted for the water tube. The smoking apparatus allowed a monkey to smoke lit cigarettes by automatically lighting each cigarette, spacing the cigarettes over time and sensing changes in pressure as the monkey puffed. When the monkeys had learned to puff, they were so trained that they had to puff but were allowed to choose between smoke and air⁶. This procedure was developed to reduce

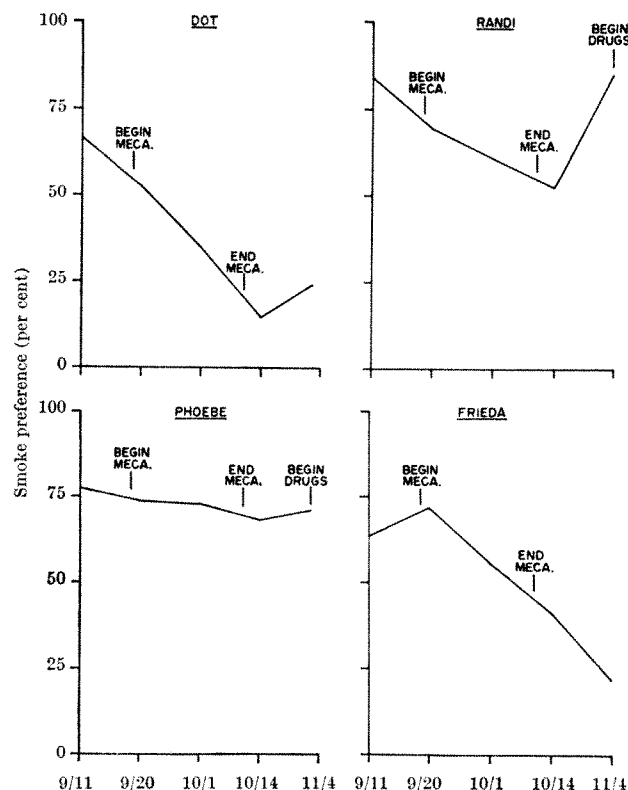


Fig. 1. Changes in smoke preference on non-drug days induced by mecamlamine.

the large variability from subject to subject inherent in free puffing.

A smoking apparatus was attached to a 'Plexiglas' panel covering a 14 inch square opening in the door of each monkey's home cage. Two tubes, one delivering cigarette smoke and a "dummy" tube with access only to air, were also mounted on the panel. A water solenoid was mounted between the smoke and air tubes. A puff on either the smoke or air tubes preset the solenoid and would release a small amount of water when the monkey touched the solenoid spout. A light mounted above the solenoid signalled the availability of water. A fixed ratio (FR) contingency was added, so that a monkey could be required to puff a specific number of times to obtain water.

The monkeys received all their water during a daily 4 h puffing session. Thirty cigarettes were loaded into the smoking apparatus each morning and the test was started at 1030 h. A new cigarette was automatically lit every 7.5 min. The positions of the smoke and air tubes were interchanged each morning so that side preferences would not develop. Initially, water could be won with a single puff on either the smoke or air tube. The number of puffs for a reward of water was then increased to five, ten, twenty and finally to thirty. The FR 30 schedule was then maintained. Puffs were recorded on Sodeco counters and on an Esterline Angus recorder.

All four monkeys preferred smoke to air; that is, although they could get water by puffing on either the smoke or the air tubes, they reliably made more puffs on the smoke tube than on the air tube. When puffing rates on the FR 30 schedule stabilized, drug administration began.

The first drug was mecamlamine which is known⁷ to block both the peripheral and central effects of nicotine. Mecamlamine, in doses ranging from 0.4 to 3.2 mg/kg, was given intramuscularly 15 min before the beginning of a puffing session. Each dose was repeated two to five times, with at least 3 days between successive injections.

The results obtained from mecamlamine administration are shown in Table 1. The data on days immediately

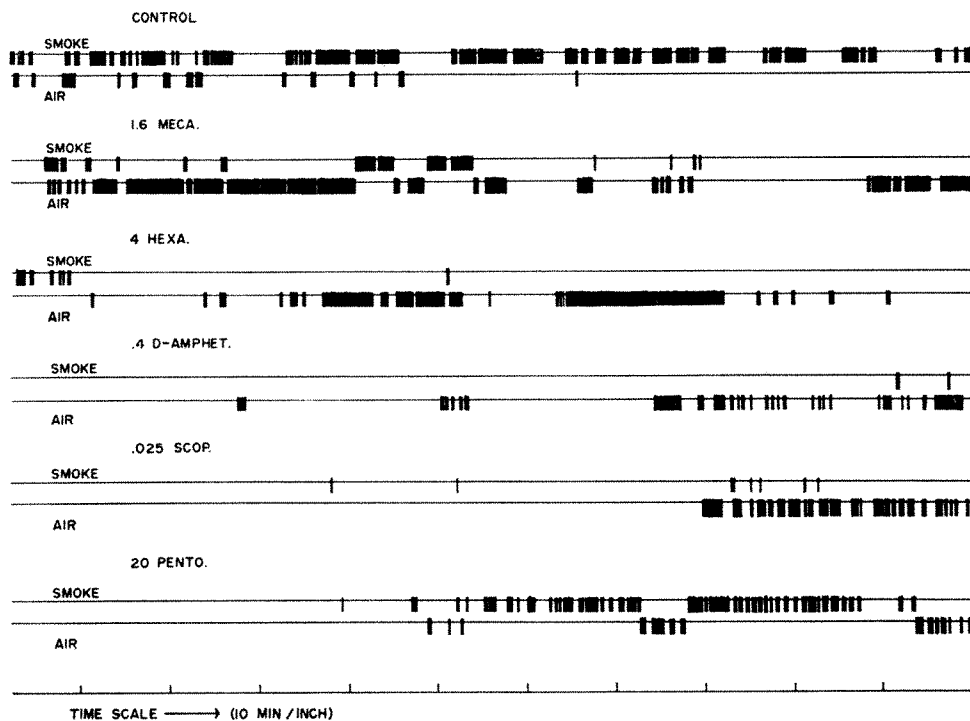


Fig. 2. Typical Esterline Angus puffing records.

preceding injections were used as control data in paired *t* tests. For each monkey, a dose of mecamlamine was found which reversed the smoke-air preference while producing a small decrease in the overall puffing rate.

We had not expected that mecamlamine would make smoke less desirable than air. If mecamlamine blocks the incentive effect of nicotine, then one might reason that smoke and air should be equally rewarding or that smoking should increase in order to get more nicotine and overcome the block. The reversal suggests that nicotine is the rewarding component in cigarette smoke and that smoke without nicotine is aversive. Mecamlamine, by blocking nicotine, may leave only the aversiveness of smoke itself.

It took approximately a month with two injections a week to complete the course of mecamlamine administration summarized in Table 1. During this time a gradual and consistent change in the smoke-air preferences under non-drug conditions was noted. The smoke preferences of the monkeys decreased and, after the last injection of mecamlamine, two of the monkeys still preferred air to smoke. Fig. 1 shows this progression for each of the monkeys, two of which recovered their preference for smoke. The other two monkeys, whose initial preferences were lower than those of the first two, did not recover within a 1 month period of observation. This long term after-effect of mecamlamine administration could be ascribed to negative conditioning; that is, repeated experience with smoke as an aversive stimulus precluded the rewarding effect of nicotine.

Table 1. CHANGES IN SMOKE PREFERENCE INDUCED BY MECAMLAMINE IN MONKEYS

Dose (mg/kg)	Dot		Randi		Phoebe		Freida	
	Total puffs	Per-centage smoke pref.	Total puffs	Per-centage smoke pref.	Total puffs	Per-centage smoke pref.	Total puffs	Per-centage smoke pref.
Control	4,461	64.6	4,274	82.5	4,515	76.7	2,821	70.8
0.8			3,658	84.3	4,834	67.7		
1.2			3,953	64.4*	3,437	35.7*		
1.6	3,795	70.3	3,757	1.7*	3,327*	12.3*	2,955	71.7
2.4	3,002	69.3					1,907	69.1
3.2	2,867*	39.3*					1,765*	33.5*

* Significantly less than control at $P < 0.05$.

Table 2. DRUG-INDUCED CHANGES IN SMOKE PREFERENCE IN MONKEYS

Drug	Dose (mg/kg)	Randi		Phoebe	
		Total puffs	Percentage smoke pref.	Total puffs	Percentage smoke pref.
Control		4,618	82.7	4,537	74.6
Pentobarbital	10	4,496	87.1	2,822*	78.6
	20	3,514*	68.8	2,635*	77.4
	30	2,028*	71.6	1,347*	78.7
Hexamethonium	1	4,491	80.0	4,016	80.9
	2	4,492	78.8	3,821	75.7
	4	4,243	27.4*	4,092	80.5
	8	2,084*	1.0*	4,008	69.6
	12			3,013*	39.4*
Scopolamine	0.025	2,043*	19.6*	2,902*	65.3
	0.05	741*	38.6*	3,136*	76.5
	0.1			2,530*	55.6*
	0.2			1,554*	44.7*
D-Amphetamine	0.2	1,614*	71.3	3,813	66.6
	0.4	114*	24.2*	1,143*	14.8*
Mecamlamine	1.6	3,844	14.1*	4,553	15.2*

* Significantly less than control at $P < 0.05$.

When the smoking preferences of the two recovered monkeys stabilized, the drug experiment was continued with them. Doses of pentobarbital, hexamethonium, scopolamine and D-amphetamine were given, in that order. One final dose of mecamlamine was also given. The results obtained from this series of injections are shown in Table 2. Pentobarbital, a barbiturate anaesthetic, decreased the overall puffing rate but did not change the smoke-air preferences. Hexamethonium, a nicotinic-blocker in the periphery which does not readily enter the brain⁷, reversed the smoke-air preference with large doses while decreasing the overall puffing rate. Scopolamine, a cholinergic blocking agent, and D-amphetamine, an adrenergic agonist, also reversed the smoke-air preferences and overall puffing rates were greatly decreased. Mecamlamine, again, reversed the smoke-air preferences with a small decrease in overall puffing rates.

The pentobarbital results indicate that a preference reversal is not just an artefact caused by decreased overall puffing. The hexamethonium results suggest that a peripheral action of nicotine may be part of the smoking incentive. Alternatively, in view of the much lesser potency of hexamethonium as compared with mecaml-

amine, perhaps a small amount of hexamethonium entered the brain.

A comparison of the patterns of puffing with the various drugs suggests that the scopolamine and D-amphetamine results may have been an artefact of decreased overall puffing. Fig. 2 shows sample patterns of smoke and air puffing under each of the drugs. Puffs are distributed fairly evenly in non-drug conditions and with mecamlamine, hexamethonium and pentobarbital. In contrast, puffing with scopolamine and D-amphetamine was completely suppressed for at least the first hour of the test session. Puffing on the air tube with these two drugs may have been a rebound after-effect caused by increased thirst when the drugs had been physiologically eliminated. To confirm this interpretation, we ran a deprivation test. A daily puffing period without drugs was started 2 h later than usual. Both monkeys had reversed smoke-air preferences at the beginning of the test sessions. Increased thirst may thus make smoke more aversive.

The results of this experiment must be qualified because of their questionable correspondence to human smoking. The forced and restricted nature of the monkeys' puffing certainly bears little resemblance to the free and intermittent smoking by humans. The ultimate importance of the present results will depend on their therapeutic significance. If, as these data suggest, nicotinic-blocking drugs will inhibit smoking by humans, then the animal paradigm will have been a most useful one.

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Belief in the Supernatural among Harvard and West African University Students

JAHODA¹ has assessed the effect of university education on traditional supernatural beliefs held by many students at the University of Ghana, and concluded that there was no evidence that education, "including scientific training, had any discernible impact on the magico-mythical beliefs". He therefore suggests that if such beliefs impede the growth of science in developing countries, methods other than general education must be used to diminish them. The growth of a superstitious sub-culture now apparent in the United States, including increased popular interest in horoscopes, and our desire to understand better the African studies, led us to attempt a similar investigation on undergraduates at Harvard University.

Two hundred male students at Kirkland House of Harvard College answered, on a scale from 0 to 20 chosen to match Jahoda's, a questionnaire containing nine questions. With instructions explaining that 0 indicated "total unqualified disbelief", 10 indicated "neither belief nor disbelief", and 20 indicated "total unqualified belief",

they were asked whether they believed in astrology, extra-sensory perception, flying saucers, and the power of prayer. Two questions, whether science could ever predict "human actions" and "all physical occurrences", were set aside because of ambiguous phrasing. We also asked the students whether they ever crossed their fingers to bring themselves luck, "knocked on wood", or "walked under ladders", with 0 indicating "never" and 20 indicating "always". We believe that the selection effect is negligible (because essentially all those having dinner on a certain night in January 1969 were polled), and that the students were answering honestly. The smallest number of students who answered each of the questions, and their distribution by year and field of study, are shown in Table 1. Because not all respondents answered quite every question, and because the table headings omit the three freshman, five staff members, and five who answered "other" to major field, the column totals are not exactly the weighted averages of the subcategories.

Table 1. MINIMUM NUMBER OF STUDENTS ANSWERING EACH QUESTION

	Second year	Third year	Fourth year	All years
Humanities	10	14	4	33
Social sciences	33	28	23	90
Natural sciences	13	6	9	32
Biological sciences	11	5	6	24
All fields	71	58	43	194

The average belief in astrology was only 2.5 on our scale of 20, extra-sensory perception was as much as 11.9, flying saucers was 8.5, and the power of prayer was 8.0. Thus many students at Harvard do not reject the supernatural, although they do not accept such fixed systems of belief as astrology. Table 2 shows the distribution of averages by year in university and by major field of concentration. Even for astrology, for which 161 students indicated disbelief by giving assessments of 5 or less, sixteen students gave neutral scores of 10, and nine students, almost 5 per cent, assessed their belief at 15 or better. Only 35 per cent were nearly certain that flying saucers do not exist, with 30 per cent neutral and 20 per cent strongly believing.

Table 2. AVERAGE BELIEF ON A SCALE FROM 0 TO 20

	Second year	Third year	Fourth year	All years
(a) Do you believe in astrology, that the stars affect our actions on Earth?				
Humanities	3.5	4.0	3.8	4.5
Social sciences	2.8	0.9	2.7	2.0
Natural sciences	0.2	2.0	2.0	1.2
Biological sciences	1.6	0.0	3.0	1.5
All fields	2.3	2.1	2.6	2.5
(b) Do you believe in extra-sensory perception (ESP)?				
Humanities	13.5	12.0	13.7	13.0
Social sciences	13.8	11.1	12.4	12.8
Natural sciences	9.6	11.0	7.5	8.9
Biological sciences	12.1	8.0	10.5	10.9
All fields	12.6	11.0	11.2	11.9
(c) Do you believe in flying saucers?				
Humanities	10.0	7.2	8.0	9.0
Social sciences	12.0	0.6	8.9	9.3
Natural sciences	6.4	9.0	4.9	6.1
Biological sciences	6.9	7.0	8.8	7.7
All fields	9.6	6.9	8.0	8.5
(d) Do you believe in the power of prayer?				
Humanities	5.7	7.0	12.5	7.8
Social sciences	9.7	7.8	7.7	8.7
Natural sciences	6.2	4.0	6.9	6.0
Biological sciences	11.5	3.4	9.2	9.5
All fields	8.4	6.7	8.0	8.0

Students of the natural sciences believed markedly less than their fellows in astrology, extra-sensory perception and flying saucers. Assuming a standard error of about 2, there are no clear differences between students of the humanities and social sciences. Natural scientists also seem less believing in the power of prayer, although the fluctuations in these data are higher and hence the conclusion is less significant. It is interesting that students of the biological sciences tended to have higher belief scores than their natural science peers. There is no obvious correlation with number of years of university education.

The students replied that they almost never crossed their fingers to bring themselves luck or "knocked on wood", with assessments of 2.7 and 3.5, respectively. The symmetrical distribution of answers to the question about walking under ladders, with an average score of 10.3, may reflect an ambiguity in the wording of the question, although it could also reflect a cultural habit.

Thus a sizable fraction of the well educated students under study do not reject beliefs in supernatural explanations, though the scientists tend to be somewhat more sceptical. The overall level of superstition is sometimes substantial among Harvard students. At the University of Ghana, where Jahoda¹ found "mean belief scores" between 5 and 8 out of 20, his data showed that the science students did not differ from the overall sample, as they tended to at Harvard. Of course, we asked different specific questions and evaluated replies in a different manner, so our study cannot be used to provide an American baseline for his work.

We thank the master and senior tutor of Kirkland House, Harvard College, for their cooperation, and Professor Owen Gingerich and the Smithsonian Astrophysical Observatory for their assistance with computing facilities.

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"Put Away"—For How Long?

MORRIS¹ has stated that "In subnormality hospitals . . . most patients, once admitted, will never leave." In the past this was true, but the present trend is for subnormal individuals to remain at home, adequately supported by appropriate services, for as long as possible. Moreover, it has been shown that 10 per cent or less² of populations of subnormality hospitals require "detailed specialist day to day diagnosis, care and treatment"³. 80 per cent of severely subnormal children now remain at home⁴. One of the services which can extend the time for which families can tolerate a mentally handicapped dependant at home is periodic short term care in hospital. Of all admissions to mental subnormality hospitals and units in England and Wales, 48 per cent in 1964 and 60 per cent in 1966 were for informal short term care, usually for eight weeks or less⁵. We present here a short account of a study aimed at discovering whether the same factors determine short term and long term care for children and fulfil the same needs.

In this hospital during 1964-66 it was possible to offer either short term or permanent care almost equally readily; of 373 admissions, 58 per cent were for short term care. Subsequently, there has been almost no option for long term care, irrespective of the need. After exclusions (such as multiple admissions), 285 records were studied, and the results analysed by computer.

Of the 164 short term care children, only 9 per cent remained in longer than eight weeks; of the long term admissions, 5 per cent remained in for less than eight weeks, and 89 per cent remained over a year. Although more boys than girls were admitted, as has been observed elsewhere^{6,7} the same proportion of each sex came in both for short or long term care. Penrose⁸ thought that probably society was less able to tolerate inadequate role performance among males; fortunately it seems that, if so, the in-

tolerance is as likely to be temporary as permanent. Similarly, there was no significant difference in the age on admission of the children who came in for long term or short term admissions; 46 per cent of the short term and 41 per cent of the long term care patients were between 1 and 4 years, and 21 per cent of each type of care were between 4 and 7 years old.

110 of the children, 43 per cent of those formally psychologically assessed, were profoundly retarded (IQ below 20) and a further 127 (49 per cent) were severely or moderately retarded (IQ 20-51), but as the age on admission increased, so did the proportion who were profoundly retarded: 62 per cent of 37 children admitted under the age of 1 year, 67 per cent of 116 admitted between 1 and 4 years; 89 per cent of 54 between 4 and 7 years; and 85 per cent between 7 and 10 and 10 and 13 years were profoundly retarded according to tests.

When the reasons for which hospital care had been requested were considered, significantly fewer of the short term care children were reported to be illegitimate or to present difficulties of management in the home, or to have serious physical disabilities (excluding spastic cerebral palsy or epilepsy, which occurred in approximately one third of both groups). An assessment of the parents' health indicated that maternal physical illness was associated with 42 per cent of the short term admissions, but with only 24 per cent of long term admissions.

Physically, approximately three quarters of both groups were in good general condition, requiring no active medical treatment. Of those measured⁹, four fifths of the children were below the average for their age in height, and three quarters were below average in weight, in both groups. The most commonly seen physical defect was squint, which in varying severity was noted in one quarter of both groups, although the general population incidence has been estimated as 1-2 per cent¹⁰. Emotionally, in both groups less than a tenth of the children were overactive, destructive, aggressive, self-mutilating or excessively attention-seeking.

We conclude that at the present time over half the patients admitted to subnormality hospitals will leave again within a few weeks. Objectively, there is little to distinguish those who leave from those who do not. It therefore seems that community care, backed where necessary by short term residential care, will increasingly become a superior alternative to long term residential care for severely mentally handicapped children, and few indeed will "never leave".

We thank Mr Eisenberg, Mr Duckor and Miss Meredith for their help; the Institute of Computer Science for the use of the Atlas Computer of the University of London; and the South West Metropolitan Regional Hospital Board for their research grant which enabled us to secure the help of Mrs Edlin.

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Book Reviews

BELOW THE SEA FLOOR

Initial Reports of the Deep Sea Drilling Project

Vol. 1: Orange, Texas to Hoboken, NJ, August–September 1968. Pp. xx + 672. \$10.25. Vol. 2: Hoboken to Dakar, Senegal, October–November 1968. Pp. xx + 490. \$8.25. (Prepared for the National Science Foundation, National Ocean Sediment Coring Program by the University of California, Scripps Institution of Oceanography.) (US Government Printing Office: Washington DC, February 1970.)

On August 12, 1968, the drilling vessel *Glomar Challenger* began boring its first hole in the Gulf of Mexico. Compared with the aims of the Mohole project, which was finally abandoned two years earlier, the aims of the Deep Sea Drilling Project were modest: the acquisition of information pertaining to the history of the ocean basins.

These two splendidly produced and illustrated volumes cover the first two legs of the project. On the first leg various holes were drilled in the Gulf of Mexico and the eastern North Atlantic; the second group of drillings took place in the North Atlantic on a line between New York and Dakar. The volumes contain excellent descriptions of the holes drilled and the rocks found in them; but are written mostly in technical language, with the detailed results on which any conclusions are based. They will provide a superb foundation for future work on the drilling cores, but neither they nor the shorter cruise reports of later legs convey any of the excitement which the project has generated. Nor do they (and indeed nor should they!) make any attempt to discuss the wider implications of the results of these studies.

Before this project began little was known about the sediments and volcanic rocks below the sea floor. The soft surface sediments had been cored to a depth of 10 m in many places, and loose fragments of basalt and other basic and ultrabasic rocks had been dredged. The age and composition of the huge wedges of sediment which extend from the continental slopes beneath the abyssal plains were, however, quite unknown. Before samples of these rocks had been obtained, many geologists claimed that the few rocks now exposed on the continent had been deposited in the deep ocean basins. As soon as cores of these rocks were obtained, however, they were found to be remarkably similar to the rocks which form some major mountain belts.

Perhaps the youth of the ocean basins and their economic importance are the two most surprising and important results of the deep drilling programme. When drilling began in 1968 the theory of sea floor spreading had just become accepted by most geophysicists, and large parts of the world's oceans had been dated by using the characteristic shape of magnetic anomalies observed at the sea surface. There was, however, rather little evidence to support the reversal time scale used for this purpose. The spreading rates obtained from the magnetic anomalies also required almost all the ocean floor to have been produced in the 200 m.y. since the Jurassic, or in the last twentieth of the geological record. Therefore if these results were correct the ocean floor had to be very much younger than the continents, where rocks of 3,000 m.y. have been found in several regions. The deep drilling results have supported all these suggestions in the most remarkable way. Particularly impressive is the comparison

of the dates obtained from microfossils just above the basalt in the South Atlantic with the dates from the magnetic anomalies¹. The agreement between the two is remarkable, and removes any lingering doubts about sea floor spreading and plate tectonics.

The other major result of this programme is the discovery of deposits of salt, sulphur, oil and copper beneath the deep ocean. A salt dome in the Gulf of Mexico was drilled during the first leg, and the rocks obtained contained a considerable quantity of oil and sulphur. The first of these volumes contains a description of the exhaustive experiments which were carried out to show that the rocks are identical in almost all respects to the cap rock of certain Gulf Coast salt domes. Seismic reflection records in the Gulf of Mexico show that there are a great many of these structures beneath the sea floor, many of which are likely to have trapped oil. Salt domes have also been found in deep water beyond the continental edge in the North Atlantic, and these also should have trapped oil. How the salt is formed is far from clear; Ewing *et al.* in volume one remark: "The regional problem which probably will be most earnestly debated is whether this salt was deposited in its present deep water environment, or on a crust which was once much shallower". Both proposals have major objections. Though there is not yet any method of exploiting the oil deposits trapped by the salt, there is little doubt that the intriguing technical problems can be overcome and the oil extracted. The quantities involved will probably equal or exceed those on land. The other major economic find is native copper, found on leg 11 in the deep ocean off Cape Hatteras. This discovery is less surprising than the salt domes because the oceanic crust consists of layered basic intrusive complexes, and most continental economic deposits of copper, chromium and nickel are associated with such complexes, some of which may be fragments of uplifted oceanic crust. The present drilling ship can only drill through a few metres of basalt, and therefore cannot hope to reach most of these ores. Perhaps it will soon be possible to revive the Mohole project under a different name and justify it on economic grounds alone. Let us hope that the exploitation of these enormously valuable deposits will not be permitted until it can be done without polluting the oceans with waste products and escaped oil.

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WEDDELL'S VOYAGE SOUTH

A Voyage towards the South Pole performed in the Years 1822–24

Containing an Examination of the Antarctic Sea (1827). By James Weddell. Pp. x + 324. (David and Charles Reprints: Newton Abbot, June 1970. First published in 1825.) 75s.

On February 20, 1823, the *Jane of Leith*, a 60 ton sealer under the command of Captain James Weddell, a retired master in the Royal Navy, reached latitude 74° 15' S, in longitude 34° 16' a point in that great bight of the Antarctic continent which Weddell named for George IV, but which was soon to become better known as the Weddell Sea. He was only 945 miles distant from the South Pole and virtually open water lay in all directions. This remarkable southing beat Cook's previous record of 71° 10' S and was itself not to be surpassed in this sector until 1912 when the German, Wilhelm Filchner, attained 77° 44' S in the *Deutschland*. It marked an epoch in the history of Antarctic exploration by pointing to one of the principal routes to the far south, but one that in most seasons is so ice infested that few vessels can penetrate it. Dumont d'Urville, Wilkes and Clark Ross

all subsequently failed in their efforts to surpass Weddell's achievement. Filchner's Deutschland and Bruce's Scotia narrowly escaped disaster and Shackleton's Endurance drifted for ten months before finally succumbing to the almighty pressure of its pack. This was Weddell's second voyage to the Antarctic and it was, by no means, the first to harvest fur seals in the waters south of Cape Horn. Only a few years previously, in 1819, another British sealer, William Smith, had revealed the riches of the South Shetland Islands. But these early sealers were a secretive lot, not given to keeping logs let alone writing books.

Weddell's narrative, first published in 1825, the second edition (1827) of which David and Charles have now reprinted at a tenth of its current auction room value, is of singular interest. It combines one of the very few published accounts of the Antarctic sealing industry at this period with numerous observations on the natural history of the region by an amateur with all the makings of an objective scientist. The text is illustrated with careful charts of the track of the Jane and the Beaufoy (Captain Brisbane), which accompanied her; of Tierra del Fuego, the South Shetland Islands, and of the South Orkney Islands, visited only days after their discovery by George Powell. In addition to the textual descriptions of fauna, flora and the nature of the ice there are appended sections which include "Observations on the Navigation round Cape Horn", a discourse on the use of chronometer and tables of computed longitude. Illustrated is one of the five truly Antarctic seals discovered by Weddell and named for him *Leptonychotes weddelli*. In this enlarged edition are added "Observations on the Probability of Reaching the South Pole", in which Weddell argues the open polar sea theory, and, in conclusion, "Second Voyage of the Beaufoy to Tierra del Fuego", a very brief account of Brisbane's visit to the Fuegian Indies in 1825. The reprint is prefaced, aptly enough, by Sir Vivian Fuchs, director of the British Antarctic Survey, among whose responsibilities is the maintenance of Britain's Weddell Sea scientific station, Halley Bay, and who himself narrowly escaped Shackleton's fate when the expedition ship Theron was temporarily beset in these waters while attempting to establish an advance party for the Commonwealth Trans-Antarctic Expedition in December 1955.

H. G. R. KING

ANTARCTIC ICE

International Symposium on Antarctic Glaciological Exploration

(ISAGE), Hanover, New Hampshire, USA, September 3-7, 1968. Edited by A. J. Gow, C. Keeler, C. C. Langway and W. F. Weeks. (Publication No. 86 of the International Association of Scientific Hydrology and the Scientific Committee on Antarctic Research.) Pp. xvi + 541. (c/o SCAR, Scott Polar Research Institute: Cambridge, 1970.) 84s; \$10; B.f.50.

THIS collection of papers is an excellent representative selection of recent Antarctic glaciological research. It is generally up to date for the year of the symposium, 1968. The articles are grouped under thirteen sections and include presentations of technique, theory, data and general reviews. The emphasis is on the historical and regional aspects of mass ice balance, glacial meteorology, and "deep sounding", which includes deep core drilling, deep probing, and radio echo and seismic exploration. The increasingly important field of glacio-chemistry is represented in two sections devoted to studies of accumulation and particles. Analyses of the dynamics and thermodynamics of ice sheets are presented in two rather short sections. There are also sections devoted to fringe regions of the ice sheet, ice shelves and sea ice. The latter two topics are singled out, along with the ice-rock interface, as promising frontiers of Antarctic glaciology

in the frank and witty presidential address by A. P. Crary.

The great impact of the relatively new techniques of deep core drilling and radio echo sounding on Antarctic glaciology is very well shown in the "deep sounding" sections and many new applications of these tools are suggested. Even mishaps can sometimes be put to good use, as in J. Weertman's estimate of minimum water layer thickness from upwelling into a borehole. The eclectic nature of glaciology and the proliferation of sub-disciplines is impressed upon the reader, as suggested by the enumeration of subjects earlier.

Among the highlights of the symposium are the results of the studies of ice structure in the cores from the deep drill hole at Byrd Station by A. J. Gow, the detection of reflecting horizons within the ice by aerial radio echo sounding over a wide sector of Antarctica by G. de Q. Robin, *et al.*, movement studies of the Ross Ice Shelf by C. Swithinbank, and review articles on mass budget of the ice sheet as a whole by P. A. Shumsky and J. Hollin.

While most of the articles are complete, a few are only abstracts. The figures and tables are consistently pointed and readable; bibliographical completeness varies with the author. This volume will be of great value to glaciologists, but there is also much of interest to geophysicists, meteorologists, oceanographers, Pleistocene geologists and students of the polar regions in general.

GILBERT DEWART

PULSATIONS ABOVE THE EARTH

Geomagnetic Micropulsations

By J. A. Jacobs. (Physics and Chemistry in Space, Vol. 1.) Pp. viii + 179. (Springer-Verlag: Berlin and New York, 1970.) 36 DM; \$9.90.

ANYBODY who has ever had the need to browse through issues of the old *Journal of Terrestrial Magnetism and Atmospheric Electricity* published during the 1920s and 1930s will know that they were devoted almost exclusively to magnetic and electrical effects above the Earth's surface. Indeed, the few per cent of the Earth's surface field produced externally attracted attention in inverse proportion to its strength. This state of affairs has changed radically over the past twenty years; but, palaeomagnetism notwithstanding, it is still true that more papers are published on the external than the internal field. While investigations into the magnetic field produced inside the Earth have received much of the scientific limelight in recent years, studies of the external field have been going from strength to strength, notably since the advent of the artificial satellite.

Professor Jacobs's new book is concerned with one small aspect of the external magnetic field—geomagnetic micropulsations, those fluctuations of the field whose amplitudes range from a fraction of a gamma to, occasionally, as much as a few tens of gammas, and whose periods lie between 0.1 second and 10 minutes. It is not a review of all the work that has ever been carried out in this field but rather a summary of our present state of knowledge, which means, roughly speaking, a concentration on the progress made during the past decade. A long section on the morphology of geomagnetic micropulsations is followed by a consideration of magnetohydrodynamic waves, the basis of modern theories of micropulsations. This leads naturally into the theories themselves. The final, and most interesting, chapter discusses the insight that studies of micropulsations have given us into the magnetosphere itself. As Jacobs himself points out, one of the most exciting aspects of the subject is the way that micropulsations are being used, and will be increasingly used, for remote sensing of the magnetosphere.

Geomagnetic Micropulsations would be classed as a research monograph (and a good one), which means that

it would be tough reading for a newcomer to the subject. The choice of approach is, of course, Jacobs's prerogative. Even so, I would like to have seen a somewhat longer introductory chapter discussing in greater detail the philosophy and nomenclature of the subject. Jacobs is, however, to be congratulated on sticking to cgs units throughout. It really is quite monstrous that attempts should be made to force all scientific disciplines into using a system of units which may be appropriate to some but not others. The tragedy is that, irrespective of whether or not geophysicists ultimately capitulate, modern students brought up on a strict diet of SI units will be largely cut off from geophysics' most glorious decades.

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INTRODUCTION TO PHYSICS

General Physics

By G. J. Aitchison. Pp. viii + 522. (Chapman and Hall: London, April 1970.) 60s.

THIS book is described in the preface as intended for students doing first year university physics courses with the probability of terminating their study of the subject at that level. Before commenting on this particular use, let it first be said that this is an extremely satisfactory book about physics, with a freshness of thought and an appeal to down-to-earth and commonsense reason which makes it something that few textbooks of physics are—readable. It is therefore a little disappointing that it moves forward to what has for a decade or two been the inevitable culmination of any treatment of “general physics”—high energy nuclear physics and the strange particles. Although this section is well done, one hopes that before long the much more open-ended climax offered by cosmology will replace this restricted field in the canon.

One is tempted to speculate whether this book would not be an admirable introduction to the subject for a very large number of able people who would like to understand something about physics, but have never followed it beyond school days, at least as part of their formal education. It has the qualities of a book which can be read and thought about at the pace which suits the reader, and with a little determination to follow the author's advice and do all the examples, the exercise could hardly fail to be profitable.

As a first year textbook, it has the same excellent qualities, but for this purpose it would be necessary to shape the courses to be compatible with the aims and methods of the book. A general impression is that rather more time would be needed than is sometimes allocated to a “subsidiary” subject; moreover, any examination on the course would have equally to be related to the author's view.

Here is a book which makes physics neither remote nor mysterious, and it deserves to do well.

J. G. WILSON

CONDUCTING STATES

Amorphous and Liquid Semiconductors

Edited by Sir Nevill Mott. (Proceedings of the International Conference held at the Cavendish Laboratory, Cambridge, September 24–27, 1969.) Pp. xii + 626. (North-Holland: Amsterdam, 1970.) Hfl. 108; 252s; \$30.

THE editor and everybody concerned must be congratulated for producing this very valuable volume in record time, though a penalty is paid in many printing errors. Interest in amorphous and liquid semiconductors has grown rapidly in the past few years, but our knowledge of

them is still in its infancy, so that many of the contributions are in the nature of pioneer work.

Even if some of these substances have important technological applications, the theory is of predominant interest at this stage. It is much more complicated than for crystalline semiconductors and its principal aim is to discover the differences between regular and disordered structures in the electronic energy spectrum and in the character of the energy states, the problem of the localization of these states being in the forefront. The very clear review paper on the theory is by Morrel Cohen, who points out what may be universal features of the electronic structures of disordered systems. He discusses the model which has emerged largely through the work of Sir Nevill Mott. An isolated energy band in a disordered material is assumed to consist of extended states between two critical energies, with tails of localized states outside these energies. Tails from neighbouring bands may overlap. The transport mechanism also changes at the critical energies E_c ; for the extended states it is similar to that in crystals, but, because the carriers are almost continuously under the influence of the scattering centres, their motion is more like a Brownian motion than a wave propagation; for the localized states we have phonon assisted hopping. The mobility changes by several orders of magnitude within a range of order kT about E_c , thus it is more appropriate to speak of a mobility edge and mobility gap rather than of an energy gap.

S. F. Edwards explores, in an elegant paper, the analogy of the Mott transition with a phase change of a thermodynamical system. He establishes a relation between the density of states of the electrons in a disordered system and the thermodynamics of a flexible chain, so that the problem is reduced to counting the configurations of this chain. The result is that a phase change can indeed take place. It is not yet certain whether the conductivity drops to zero quite abruptly.

The title of the last section, “Non-ohmic Behaviour of Semiconducting Films and the Phenomenon of Switching”, brings to mind the work of S. R. Ovshinsky, and he himself is in fact co-author of five articles. In one of them, with H. Fritzsche, he discusses the conducting state in covalent alloy semiconductors which is maintained only above a certain value of the current, and the various processes that can trigger this high field breakdown. Another contribution by H. K. Henisch, E. A. Fagen and Ovshinsky discusses switching in monostable structures in terms of a double injection space charge model.

It is a pity that in a short review it is impossible to mention several other important contributions. In brief, we have here a collection of absorbing descriptions of the early exploration of a vast field.

L. PINCHERLE

TALK AT CORAL GABLES

Coral Gables Conference on Fundamental Interactions at High Energy 2

Center for Theoretical Studies, University of Miami, January 21–23, 1970. Edited by A. Perlmutter, Geoffrey J. Iverson and Ruth M. Williams. Pp. x + 369. (Gordon and Breach: London and New York, 1970.) \$22.50.

FOR several years now the University of Miami has been host to a winter conference on elementary particle physics organized by Professor B. Kursunoglu. The earlier meetings were restricted to symmetry physics and they provided valuable opportunities for discussion and exposition during the heady development of that subject in the middle sixties. Now that things are quieter on the symmetry front the scope of the conference has been widened to include a broader theoretical spectrum. The volume under review covers the second of the new style Coral Gables meetings held in January 1970.

The wider range inevitably results in a more fragmented and loosely connected series of talks. Moreover, two fields of much recent interest and activity—Veneziano dynamics and deep inelastic electron scattering—are only slightly treated in the proceedings. If an underlying thread can be discerned in the majority of the contributions it is probably a concern with the use of field theoretic methods. A most interesting and promising development here is represented by Salam's talk on the new methods being developed to discuss non-polynomial Lagrangian theories. Another talk which must have been most interesting is unfortunately not recorded in the proceedings, for Gell-Mann has not provided a script for his exploration of the breaking of scale invariance.

As with many conference proceedings, some of the most interesting items occur in the records of the discussions, which provide a questioning counterpoint to talks themselves. At best, however, this type of proceedings is necessarily somewhat ephemeral. The publishers are therefore to be congratulated on producing the volume with sufficient speed to conserve its utility. It is to be regretted, however, that they have set a price which would be more appropriate for a definitive treatise with expectations of longevity.

J. C. POLKINGHORNE

FUNDAMENTAL CONSTANTS

The Fundamental Constants and Quantum Electrodynamics

By B. N. Taylor, W. H. Parker and D. N. Langenberg. (*A Reviews of Modern Physics Monograph.*) Pp. xiii + 353. (Academic: New York and London, December 1969.) 47s.

THIS book, identical with an article published in *Reviews of Modern Physics* in 1969, provides a critical and comparative review of all the work bearing on the fundamental constants of quantum electrodynamics up to 1969. The original article was prompted in part by improved measurements for electrodynamic quantities (for example, the muon g -factor, the Lamb shift in deuterium, the hyperfine splitting in muonium, the fine structure of hydrogen), and improved theoretical calculations for such quantities. The strongest motivation for this reassessment, however, was probably that provided by the new possibility of determining e/h very accurately by measurement of the Josephson-effect frequency ($\nu = 2Ve/h$) for two weakly coupled superconductors maintained at d.c. voltage difference V . From the agreement found for e/h in measurements of ν in a wide variety of circumstances, the authors argue that any corrections to this simple formula are less (and probably much less) than $1/10^6$. Using the Josephson effect, they can obtain a value for $\alpha = e^2/hc$ without any essential use of quantum electrodynamics; with this value for α , they are then in a position to check empirically the quantum electrodynamic expressions given by theory, including the higher order corrections.

The book is very technical in its account of this subject, which is full of complex interrelationships, and the details discussed at length will be of interest primarily to the specialist on fundamental constants and experiments relevant to their determination. Their recommended set of fundamental constants will, however, be of more general interest, and the book includes an interesting chapter discussing what experimental and theoretical work is still needed to establish more reliably the fundamental constants. Section E ("Recommendations for Reporting Results") of this chapter could well be read by every research worker, for it illustrates the deficiencies of detail which critical readers find in many published papers today and which made the task of these authors unnecessarily difficult.

I am not completely clear why this book has been published. Its contents are readily available in a journal which will be found in every library likely to stock the book. The book has small pages, with stiff and shiny paper, which makes it tiring to read; the pages have wide margins so that the open book presents relatively little material to the eyes. I found the original article much easier to read; the journal format allows one to grasp the material more readily (each page has three times as much material) and to turn up some particular point more quickly. Perhaps republication in book form may give the article a wider attention than it may gain from the pages of a scientific journal.

The book is too detailed and technical to serve as a text. Nevertheless, its critical yet constructive approach is likely to give it an important influence on the future development of this subject.

R. H. DALITZ

GOLDEN RULES FOR ENGINEERS

Brittle Fracture in Steel Structures

Edited by G. M. Boyd. Pp. xiii + 122. (Butterworth: London, June 1970. Published for the Navy Department Advisory Committee on Structural Steel.) 85s.

THIS book sets out to present clearly to the practising design engineer the general problems associated with brittle fractures in fabricated steel structures and to give specific recommendations on how these problems may be minimized by choice of material and control of fabricating procedure. The views expressed represent the consensus of opinion of members of the Navy Department's Advisory Committee on Structural Steels (NDACSS), but to a large extent they coincide with those of the editor, who possesses a great wealth of experience in the field of brittle fracture. Any review must be written in the context that the book is meant to educate and aid practical engineers and managers, who have only a vague awareness that an "elusive phenomenon known as brittle fracture" may cause trouble in their fabricated structures.

Brittle fracture problems are initially set in perspective by describing examples of service failures up to 1965. A detailed risk assessment is made only for the case of ships and, here, I feel that it would have been particularly interesting to have speculated on risks which could be involved with the mammoth tankers and ore-carriers now being built. A summary is made of the lessons learned from experience, and the book then continues with a description of the characteristics of brittle failures, before going on to outline ways in which mechanical and metallurgical factors may affect fracture. The separate mechanical and metallurgical effects of "massiveness" could have been expounded more clearly, and, in otherwise very good sections on welding, mention should have been made of heat-affected zone embrittlement in higher alloy ("transformable") structural steels. Tests for notch ductility are described and a review is given of the methods currently favoured by different countries for selection of steel. German, Japanese and Russian practices are included, but little comment is made on the extent to which the procedures may be said to prevent service failures.

The experience and expertise of the editor and committee emerge in the chapters which give detailed instructions on design for welding; they tread cautiously with regard to the advisability of proof testing, and recommend procedures for the selection of steels. It is pleasing to see that a consensus of opinion can provide precise Charpy impact values for specification purposes, but the Charpy test is admittedly invalid as a prime reference. The point is made, but the dependence of specific Charpy figures on the background experience of steel type and application

cannot be overemphasized. They are not necessarily appropriate to the newer high strength structural steels, yet it is precisely with these that the design engineer will feel most at sea (for example, in submarine applications).

The book has rather an old-fashioned appearance, because it tends to be a summary of past experience. The NDAOSS should be urged to anticipate future trends and to consider now the recommendations that it may have to give in the future. One of the claims for the book is that it could serve as a text for engineering students; in this respect, it does not compare particularly well with certain other works on the subject, except that some of its information is more up to date. Its principal appeal is to the practical designer and, here, its job is done well for the "classical" applications: the designer is told to use his own judgement, but is provided with a watertight procedure for choice of material and a set of "golden rules" to assist his general design and construction. It is to be hoped that designers will so discipline themselves to follow the "golden rules" that they thereby prevent brittle fracture in their iron rods!

J. F. KNOTT

FLOW PHENOMENA

Separation of Flow

By Paul K. Chang. (International Series of Monographs in Interdisciplinary and Advanced Topics in Science and Engineering, Vol. 3.) Pp. xviii+777. (Pergamon: Oxford, London and New York, May 1970.) 300s; \$40.

THERE is no entirely satisfactory name for that class of hydrodynamic phenomena which includes eddying "dead-water" regions downstream of bridge piers, behind bullets, and aft of stalled aircraft wings. The term "separated flow" is often used, because it expresses the idea that the particles of fluid which travel along the front part of the body in question do not remain in contact until they reach its downstream extremity; instead, they "separate" from the body surface at some earlier station, causing its downstream parts to be swept by fluid moving in the opposite direction. Flow patterns of this kind are frequent in engineering; they may be provoked deliberately, as in the design of combustion chambers; or they may be a tiresome adjunct of other necessities, as when the wind flows past a hyperboloidal cooling tower.

The range of separated-flow phenomena is enormous, and the scientific literature about them is growing rapidly. Whoever seeks to write a textbook on the subject should therefore have read much, and have mastered diverse experimental and theoretical material; he must, moreover, be able to write about his subject sufficiently rapidly for his book not to be too out of date when it appears, to excite interest.

Professor Chang has certainly made a commendable attempt at this difficult task. He has incorporated the results of a very large number of papers into his book of nearly eight hundred pages; and (a rare bonus) he has listed many papers which, though relevant, he has been unable to quote. The book is well organized, and on each topic the author has striven to provide knowledge of experimental findings, of theoretical analyses and of practical consequences.

The principal defect of the book is that, because it has inevitably taken many years to write, some of the material is severely dated. For example, there now exist rather satisfactory computational procedures, embodied in standard computer programs, for predicting when two-dimensional laminar or turbulent boundary layers will separate from a solid surface. Chang does not mention these, but gives space, instead, to methods which, though they enjoyed a certain vogue ten or fifteen years ago, are now largely discredited. The author has accomplished so much, that defects of this kind are no disgrace to him;

it is nevertheless the duty of a reviewer to point them out.

The book is handsomely produced. The style of the English is not entirely free from signs that the author is writing in a language which is not his own, but it reads smoothly enough. The greatest benefit that the book brings, however, is that it is a unique compilation of information old and new, on an important technological subject; many research workers will find it a useful guide to the literature.

D. B. SPALDING

REMOTE SENSORS

Synthetic Aperture Radar Systems

Theory and Design. By Robert O. Harger. (Electrical Science Series.) Pp. xiii+240. (Academic: New York and London, January 1970.) 126s.

THE synthetic aperture radar (SAR) system is primarily used to form an image of terrain from a remote location. It is a scanning system which overcomes the physical constraints which limit the more straightforward radar systems. The along-track resolution does not depend exclusively on the narrowness of the scanning beam, but is provided by Doppler processing the received echoes. The basic idea originates in the work of Carl Wiley of the Goodyear Corporation during the early 1950s. He observed that there was a one-to-one correspondence between the along-track coordinate of a reflective object being linearly traversed by a radar beam and the instantaneous Doppler frequency shift of the signal reflected by the object. A finer along-track resolution was obtained by a frequency analysis of the reflected signals than that allowed by the along-track width of the physical beam. The SAR consists of a transmitter, receiver, processor, and associated storage device. A stable oscillator serves as a common reference source for both transmitter and receiver, that is to say, the radar is coherent. The simplest data processor is an optical processor, which, in effect, is an analog computer carrying out its calculation by physical operations which very closely copy the actual generation of the radar data from the terrain the image of which is to be recovered.

The author of this book was, until recently, a member of the Radar and Optics Laboratory of the University of Michigan, where the first successful demonstration of SAR was performed in 1953 and where, in more recent years, the optical system signal-processor has been developed. The book is based on lectures prepared for the intensive course on SAR given annually since 1966 at the university, and is written on three levels. The first two chapters discuss in a simplified and general manner the principles, important parameters, and elementary system design of SAR systems. On the second level of discussion, the next six chapters deal with the optimum design of such systems. The basic limitations and sources of error are examined in some detail. The effects of phase errors, nonlinearities, motion errors and the scattering mechanism on the systems are considered. It is shown that the "ambiguity function" of the SAR is not the same as the "classical" function for a range-Doppler radar, and, that unlike the "classical" radar, the SAR possesses an inherent suppression of range ambiguities. Finally, in the last two chapters, the influence that the effects of the error sources should have on the design of SAR systems is discussed. The requirements are far more complicated and less flexible data processing.

Throughout the author gives a very clear exposition. The language of system studies is mathematical and the reader should have considerable knowledge of the theory of radar, the basic elements of matched filtering and pulse compression, optical processing and even holography. This is a pioneer and authoritative text. Each chapter concludes with valuable comments and references, and

reflects in its presentation the excellent manner in which the lecture material must have been prepared and delivered. The imaging radar system is an interesting remote sensing technique which should find application in many branches of science and engineering, particularly those concerned with earth resource surveying. No electronic circuits appear in the book, only block and optical diagrams. The student of physical optics will find much which he recognizes, and, indeed, the author pays ample tribute to the contributions of Professor E. N. Leith to the understanding and success of SAR. This is a book to be recommended to the serious student of system design engineering and particularly to those who are engaged in current research in the field of high-resolution radar.

S. WEINTROUB

COMPLEX VARIABLES

Functions of a Complex Variable

By D. O. Tall. Vols. 1 and 2. (Library of Mathematics.) Vol. 1: Pp. 72. Vol. 2: Pp. 80. (Routledge and Kegan Paul: London; Dover: New York, July 1970.) 18s boards; 9s paper (each).

This subject is one of the pleasantest in the undergraduate course. Theorems are easy to comprehend, though not always to prove, and applications are varied and fascinating; as Littlewood has said, everybody is struck on first meeting with the calculation of definite integrals by contour integration, and to this one might perhaps add conformal mapping.

The author assumes a knowledge of complex numbers, such as may be obtained from Ledermann's book in the same series, and is therefore able to discuss straightaway the topics of differentiation, integration and Taylor series. When topological properties are involved, these are clearly stated but proofs are generally omitted; most students at this stage will be prepared to accept, for instance, Jordan's curve theorem as a reasonable assumption. Cauchy's theorem is proved by means of the Stokes formula, but a proof under less restrictive conditions is given for a triangle; the step from here to the general result is again a matter for topology.

The second volume deals with applications. There is a somewhat brief introduction to conformal mapping and harmonic functions. Then we have a good account of the calculus of residues and applications, particularly to definite integrals; here the author deals carefully with principal values. The final chapter concerns analytic continuation and Riemann surfaces. In twelve small pages, only a very cursory account can be provided, and its chief use should be to whet the reader's appetite for further study.

Exercises are kept at an elementary level, and are not so numerous as to daunt the student; solutions are supplied.

T. A. A. BROADBENT

DATA FOR TOXICOLOGISTS

Handbook of Analytical Toxicology

Edited by Irving Sunshine. Pp. xiv + 1081. (Chemical Rubber: Ohio; Blackwell (Scientific): Oxford, 1969.) 280s.

This book is claimed to be a collection of data essential to scientists engaged in the analysis of drugs, poisons, and industrial chemicals. It is compiled on similar lines to the well known *Handbook of Chemistry and Physics*, from the same publisher. Types of compounds covered include drugs (the largest group, occupying about 550 pages), pesticides, described as economic poisons (113 pages), industrial chemicals (confined to those for which American threshold limit values are available) (98 pages), and smaller sections on air pollution and water analysis.

The drugs selected are those considered to be of greatest

interest to toxicologists, and inorganic compounds are excluded. Much useful data are given on toxicity, metabolism and excretion, isolation procedures and identification. Sections are included on micro melting point determinations and microcrystal tests. Ultraviolet absorption data are given for many drugs and spectra are reproduced for 290 compounds. Infrared spectra, with tables to enable many pharmaceuticals and pesticides to be identified through the well known Sadtler collection, are also provided. Large sections are devoted to chromatographic data (paper, thin layer and gas), giving R_F values or retention times in many systems, and details of detection reagents. Physical methods of analysis are discussed, including infrared, fluorescence and atomic absorption spectrometry, microdiffusion analysis, and chromatography.

A criticism might be that information on a particular subject, such as ultraviolet spectroscopy or chromatography, is divided among several sections in different parts of the book. This is, however, probably inevitable when so much material on different groups of compounds is collected in one volume, and in practice it is not difficult to find the required data.

Like other well known CRC publications this book is very thorough and detailed in its information. The tables are well supplied with references which greatly increases their usefulness. Many references as recent as 1968 are included. There is a comprehensive index.

The information provided is so vast that the book can hardly fail to be useful to anybody working in the field of drugs or toxicology. The book is strongly bound and well presented, which is to be expected in view of its high cost. Further editions are promised to bring the contents up to date in a field which is inevitably changing rapidly.

A. R. MATTOCKS

TOXIN TREATISE

Microbial Toxins

A Comprehensive Treatise. Edited by Samuel J. Ajl, Solomon Kadis and Thomas C. Montie. Vol. 1: Bacterial Protein Toxins. Pp. xxii + 517. (Academic: New York and London, April 1970.) 215s.

If workers on bacterial toxins have been able to complain, with justice, that there has been no book on their subject since a slim volume in 1951 and very patchy coverage by review articles, they can do so no longer. This is the first volume of a set of six which will deal with the bacterial protein toxins in three volumes, the endotoxins in two, and the algal and fungal toxins in the final one. The names of the authors of some projected chapters are a sufficient guarantee that their subjects will be dealt with adequately.

This volume provides a general survey of the protein toxins and has an admirable introductory chapter by van Heyningen, the author of the 1951 book. Then follow a chapter on nomenclature by Bonventre and two excellent chapters by Raynaud and Alouf on the kinetics of toxin formation and release, and on methods of purification and the properties of the purified toxins, followed by an equally good one on cytolytic toxins by Bernheimer. Both Bonventre and Raynaud and Alouf discuss the classification of toxins and come up with quite different answers to what is a thorny, but not fundamentally important, problem. Indeed, the freedom which the editors have allowed their authors has produced even more duplication than is usual in multi-author works and has also led to some lacunae. Thus, the discussion by Bullen of enterotoxaemia in sheep and gas gangrene in man, produced by different *Clostridium welchii* strains, makes an important point about the difficulty of determining the role of toxins in the pathology of some diseases, but is the only chapter on pathology in the book, although there is an excellent summary of recent work on toxin

pharmacology by Rašková and Mašek. One hopes pathology will be adequately discussed in later chapters on individual toxins. This is not likely to be true of immunology, the chapter on which is confined to practical methods of producing antibodies, with no discussion of the recent interesting work on the different types of immunoglobulin produced or on cellular immunity.

In spite of a few shortcomings, this volume is essential reading for workers in the field. Later volumes should be equally essential, but the total cost of £60 or £70 is likely to deter most of us from buying them all.

D. E. DOLBY

SEAWEED SYMPOSIUM

Proceedings of the Sixth International Seaweed Symposium

Held in Santiago de Compostela, September 9-13, 1968. Edited by Ramon Margalef. Pp. xxiii + 782. (Subsecretaria de la Marina Mercante, Direccion General de Pesca Maritima: Madrid, 1970.) \$25.

THIS well produced volume is almost twice the size of the proceedings of the fifth symposium and contains forty-two papers in the biological, twenty-five in the chemical and fifteen in the applied section. The majority are written in English, a few in French or Spanish. The papers are arranged in alphabetical order of authors in each section instead of a grouping by subject—the three sections presumably of the symposium, but these need not have been kept separate in the publication. The majority of papers deal with Phaeophyta or Rhodophyta, but two papers on *Scenedesmus*, one on *Skeletonema* and one on Quaternary diatoms have crept in.

Twenty years ago many papers on seaweeds were concerned with zonation on the shore, but although problems still exist in this field very few papers in this symposium deal directly with this topic. Several papers deal with light requirements, photosynthesis and/or growth of subtidal algae, for example, of *Macrocystis*, of a mixed community growing down to 130 m in the Mediterranean, another in the Canary Islands and another general account of the factors involved in coastal and clear oceanic water. Two interesting papers deal with mitotic rhythms and photoperiodic control of spore production in *Bangia* and *Porphyra*. In the latter, the strict control of life history from filamentous *Conchocelis* stage to thalloid phase is reiterated and the extensive vegetative propagation of the thalloid phase is also noted. In this paper there is a timely warning of the dangers of assuming that life histories determined under laboratory conditions are identical to those in the field. In another paper, however, the value of cultural life history studies in characterizing *Chaetomorpha* species is stressed. Two new taxa of *Porphyra* are recorded although here more cultural work seems desirable. Several papers fill gaps in our knowledge of algal distribution. The Laminariales figure again in interesting papers on *Eisenia*, *Sacchorhiza* and *Laminaria digitata*. Two papers feature transplant experiments and, from one, preliminary results show that the polymorphic *Chondrus crispus* retains its morphology under different ecological conditions. Few Rhodophytes have been artificially cultured through their life cycles and one paper is devoted to a careful study of several genera and will prove compulsory reading for anybody contemplating such research—the necessity to lower the nitrogen and phosphorus contents of the media is an interesting finding.

The papers in the chemical section are, as expected, largely concerned with the unusual polysaccharides synthesized by brown and red algae, while one paper provides evidence that the brown algae possess respiratory pathways similar to those of other plants. Papers in previous symposia have reported the seasonal changes in organic constituents in brown algae and this is now

amplified by a study of the tocopherols in these algae.

Commercial applications of marine algae are not well known to most scientists and the final section of this volume makes interesting reading and may stimulate other workers to study the pharmacological, manurial and preservative uses of seaweeds.

To summarize, this is an excellent book containing a wealth of interesting articles ranging over almost all aspects of seaweed research and illustrating the steady progress of the subject.

F. E. ROUND

NEW JOURNAL FOR ALGOLOGISTS

Algological Studies

Managing Editor O. Lhotský. No. 1. (Czechoslovak Academy of Sciences, Institute of Microbiology, Laboratory of Algology: Třeboň, 1970. Distributed outside Czechoslovakia by E. Schweizerbart'sche Verlagsbuchhandlung: Stuttgart.) DM 36.

THE Laboratory of Algology at Třeboň is well known for its studies on freshwater algae, especially the physiology and taxonomy of microscopic species and the problems involved in their mass cultivation. The laboratory has now begun publication of a new journal, *Algological Studies*, with an editor and editorial board drawn from the staff at Třeboň and an advisory council of internationally known phycologists.

The journal is primarily intended to serve the publication needs of the Třeboň laboratory, and the first number consists of papers describing work by resident and visiting phycologists. F. Hindák of the Institute of Botany at Bratislava contributes a taxonomic study of the family Ankistrodesmaceae (Chlorophyceae), especially the genera *Pseudococcomyxa*, *Chlorobion*, *Closteriopsis*, *Keratococcus* and *Monoraphidium*. Czech phycologists have a reputation for dealing successfully with these difficult chlorococcalean families and this useful paper maintains the tradition; numerous taxa are clearly described, including three new species and three new varieties, and a key to the genera is included. Next, J. Růžicka and J. Simmer describe a mathematically-based method for precise productivity measurements of autotrophic algae in cultivation, and S. Přibil and P. Marvan discuss the course of potassium uptake by cultures of *Scenedesmus quadricauda*. The following paper attempts to cover a difficult topic, that of dubious or incorrect designation of algal cultures used in experimental work: O. Lhotský reports a number of inconsistencies in the literature, especially concerning *Chlorella* but also involving species of *Chlorococcum*, *Ankistrodesmus*, *Euglena*, *Mesotaenium*, *Gomphonema*, *Anabaena* and *Polytomella*. He recommends a system of uniform designation of algal strains as suggested by Komárek in 1969. A second contribution by Hindák discusses biomass production of green algae in culture: constructional details of cultivation equipment are given and the results show that in both laboratory and field experiments a number of filamentous ulotrichalean genera (including *Ulothrix* and *Stigeoclonium*) give higher yields and simpler separation than the traditionally cultivated control, *Scenedesmus quadricauda*. This could be of great importance in commercial production. Finally, I. Šetlík, V. Šust and I. Málek describe a pilot plant for mass cultivation of algae in temperate zones in which the culture units can be used as glass-houses during the winter. Thus summer cultivation of algae is economically united with winter cultivation of higher plants, the latter probably in hydroponic units. Problems and advantages of this dual purpose scheme are considered, and the paper begins with an interesting general discussion of the principles of algal culture design.

It can be seen that this first number of *Algological Studies* is concerned with investigations of both a fundamental and applied nature and, while the primary use of

the journal will be to publish work from Třeboň, contributions are invited from other laboratories on any aspect of phycology. The standard of production of the journal is satisfactory, with clear printing on good paper. The matt finish of the paper does, however, suggest problems for studies where the results are essentially photographic. The half-tones included in the present number (chiefly photographs of experimental apparatus) are adequate for their purpose but would not be of sufficiently high quality for clear presentation of detailed electron micrographs. The chief value of the journal will doubtless be as an outlet for the rapidly increasing amount of work on algal biology in relation to the potentialities of the algae as a food source of worldwide importance.

GORDON F. LEEDALE

SPONGOLOGY

The Biology of the Porifera

Edited by W. G. Fry. (Symposia of the Zoological Society of London, No. 25.) Pp. xxviii + 512. (Academic: London and New York, May 1970.) 150s; \$22.50.

THIS new symposium in the Zoological Society series is a welcome addition. Study of sponge biology has been recently much neglected, and this collection of papers should do much to revive interest in this interesting group of animals.

The symposium, which was held in London in September 1968, was a particularly interesting one, mustering as it did, not only many of the world's authorities on sponges, but other biologists interested in cell adhesion and morphogenesis for whom sponges must have a special appeal. The papers range from fossil histories and affinities through subjects such as ecology and taxonomy of certain groups, cytology and ultrastructure. This volume is particularly welcome in that there is no modern general text dealing with sponges to which a general zoologist might turn for information, and this present work will undoubtedly provide a stimulus for further research. The principal sections are devoted to palaeontology, spicules and phylogeny, the ecology of certain groups or regions, cellular aggregation, integration of the cell types and morphogenesis. It is difficult to single out particular contributions as of particular interest for this is an individual matter. Dr Fry is to be congratulated in having achieved with such obvious success a difficult task of editing, and in producing a particularly useful index. This is a volume to be added to the shelf of every zoology department library. Certainly those with any interest in sponges cannot afford to be without it, and to those tempted into the field of sponge research, the bibliographies given in each contribution will provide a valuable starting point.

I regret that papers submitted in French or German remain in the original. These include important contributions by M. le Professeur C. Lévi, Madame O. Tuzet, Hr Dr H. Mergner, and M. le Dr R. Borojevic. It would be unrealistic to think that these chapters will be widely read by students; this is regrettable, and I think they should have been translated into English. Apart from this, Academic Press are to be congratulated in maintaining the high standard of typography and production achieved in this, as in the preceding titles of the series.

R. PHILLIPS DALES

LANGUAGE PSYCHOLOGY

An Introduction to the Psychology of Language

By Peter Herriot. (Methuen's Manuals of Modern Psychology.) Pp. 197. (Methuen: London, March 1970.) 42s.

IN recent years language has become a subject of central concern to psychologists. Some fifteen years ago it was possible to provide a survey of psychological studies of language within a coherent framework. A large range of

problems was at that time approached within a uniform associationist viewpoint; the differences between psychologists applying information theory to speech perception and those using concepts from animal behaviour to investigate how children learn to talk were chiefly ones of emphasis. This viewpoint has since been vigorously attacked by linguists for its failure to account for some of the most important properties of language. The subject matter is now in a very confused state: neither modifications to previous theories nor attempts to develop "performance models" from linguistic theory have had much success. This makes the task of writing a book today that is both an accurate survey and a coherent introduction to the subject an extremely difficult one.

Dr Herriot is one of the very few authors in recent years to attempt this. In *An Introduction to the Psychology of Language* he has produced what is a useful, accurate and well written survey of recent research on language. Perhaps, inevitably, in a book intended to be both short and comprehensive, certain sections tend to be little more than lists of technical terms, definitions and one sentence summaries of experimental studies. The range of topics discussed is a very conventional one and it is unfortunate that well known, but distantly related, work on memory is included at the cost of any reference to less familiar areas, such as bilingualism, second language learning or machine translation.

The title holds out the hope of an attempt to communicate what is exciting or challenging about the enterprise. In general, however, the author confines himself to providing a reference source, and readers for whom this book does serve as an introduction may well find the subject dull. For example, even the most critical will admit that contemporary work on generative grammars has produced fundamental and fascinating insights into language. The brief space devoted to this in the book is largely critical and a newcomer to the subject is likely to be puzzled that Chomsky and his colleagues have been so influential in the study of language. R. A. BOAKES

SELECTED TITLES

Titles in Medicine

Edited by Robert S. Goodhart. Vol. 1, No. 1. (Communico Inc.: Fairfield, New Jersey, March 1970.) \$2 per monthly issue; \$15 p.a.

IT is admirable that medical practitioners should be confronted with the existence of the medical literature and encouraged to read a little of it. This new secondary journal, which simply reprints the contents lists of about two hundred relevant periodicals, is to be sent without charge to "specific qualified physicians and surgeons" (presumably in the USA) and promises them—and other subscribers—twenty-four reprints of their choice for an annual fee of \$30.

The perceptive recipient will quickly realize that these services differ no whit from those offered by the Institute for Scientific Information's *Current Contents/Life Sciences*, which indeed would seem to be the superior (certainly handier) publication. ISI discovered that the labour of resetting contents lists could be obviated by the simple device of reproducing title pages photographically, which is not only cheaper but also more satisfying for the reader who sees the contents in their familiar format. ISI, moreover, has the grace to call its reprints "tear-sheets", thereby sidestepping some of the copyright problems thrown up by these ventures. And, finally, two hundred journals are just not enough: why not include *China's Medicine*, for example, for the benefit of those who wish to preserve a balanced outlook? And why should the practicing [sic] physician be denied a sight of titles of Letters to the Editor of *Nature*? From Vol. 1, No. 1 of *Titles in Medicine*, it seems that only the longer articles are worthy of his attention. J. H. MORRIS

Correspondence

Bode's Law

SIR,—Further to J. G. Hills's letter (*Nature*, **225**, 840; 1970) and that of John A. Antal (**227**, 642; 1970), your correspondents' comments on the following would be interesting.

Bode's law is geocentrically motivated because it uses as its basis the Earth's solar distance. It is increasingly inaccurate for planetary distances as the progression proceeds to the outer planets until by the time Pluto is reached the discrepancy is nearly 100 per cent.

However, as the table here shows, if the Jovian perihelion distance is used as a reference, it is possible to have an almost perfect prediction for planet solar distances by the simple expedient of using the arithmetical progressions of 2, 4 and 6 for the outer planets and $\frac{1}{3}$, $\frac{1}{5}$, and $\frac{1}{7}$ for the inner ones. Mercury and Pluto end the progressions with their highly elliptical orbits given by the factors 6 and 10 for Pluto's perihelion and aphelion and $\frac{1}{10}$ and $\frac{1}{16}$ for Mercury's.

Planet	Factor of Jupiter's perihelion	Perihelion/Aphelion Solar distance (million miles)	
		Actual	Predicted
Mercury	1/16	28.5	28.7
	1/10	45.7	45.9
Venus	1/7	66.7	65.7
		67.7	
Earth	1/5	91.45	91.9
		94.5	
Mars	1/3	128.5	153.2
		154.5	
Jupiter	1	459.8	—
Saturn	2	834.6	919.6
		937.6	
Uranus	4	1,699	1,839.2
		1,867	
Neptune	6	2,769	2,758.8
		2,817	
Pluto	6	2,766	2,758.8
	10	4,566	4,598

The significance of this simple arrangement is one that cannot be gone into here but the full explanation is contained in the publications *Genesis One, Two and Three*.

Yours faithfully,

D. ROOKES

c/o Ardilla Books,
29 Gloucester Crescent,
London NW1.

Library Pollution

SIR,—It has been established that the deterioration being caused by pollution can be arrested and even reversed if one is prepared to pay the cost. Cost however, needs to be related to benefit.

Deterioration may be less apparent in the case of books than in some other fields, but it is in fact insidious and the need for preservation treatment is most urgent. Moreover the wear and tear of frequent use increases the damage caused by atmospheric pollution. It is now generally conceded that excess acidity is the chief destruc-

tive agent apart from wear and tear and this acidity can arise either from present day methods of paper manufacture, when rosin alum sizes are used, or may be acquired from the sulphur dioxide content of a polluted atmosphere. So that apart from the repair of mechanical damage the deacidification of books and the prevention of further "pick up" of acid from the atmosphere are the most pressing needs.

Many effective processes for achieving these desiderata have been devised, but almost all of them are too slow and expensive to be used for the very large scale operations now necessary. As an illustration of the magnitude of the scale in question it is only necessary to cite the size of six well known and much used libraries.

According to the *World of Learning* for 1965/66 the following six libraries are believed to house about 44 million volumes distributed as follows:

	Million volumes
USA	
Library of Congress	14
University of California	6
Harvard	7
Yale	5
Great Britain	
British Museum	6
France	
Bibliothèque Nationale	6
Total!	44 million

Out of this total of 44 million volumes, assuming that half of these will be too acid to last more than 50 years, there is an obvious and urgent need to deacidify 22 million books before they deteriorate beyond repair and are lost to posterity.

The magnitude of this undertaking is enough to frighten the average librarian into *laissez faire* and this tendency will be accentuated by the apparent slowness of the deterioration which conceals the insidious nature of the damage.

If it is assumed that the 32 million volumes in the US deteriorate three times as rapidly as the 12 million of Great Britain and France (on account of the higher ambient temperature) and if a rate of deterioration in cash value equivalent to 3 pence per volume, equivalent to a useful life of 240 years for a book worth £3, is assumed, this small loss amounts in the aggregate to no less than £275,000 a year for the US alone.

Surely a small fraction of this cost spent on well directed research would lead at least to some effective first aid measures—or is the magnitude of the task too intimidating to stimulate the librarian and will it lead instead to a perpetuation of the present *laissez faire*?

There are signs that the librarians of the US are becoming alarmed at the implications of this wastage, but so far there seems little evidence that the British librarians are making any efforts to deal with this menace to our large libraries.

Yours faithfully,

W. H. LANGWELL

32 Copse Edge Avenue,
Epsom,
Surrey.

Ball Lightning

SIR,—I had just finished reading Powell and Finkelstein's article¹ and Altschuler's² chapter on Kugelblitz (ball lightning) when I came across the three recent reports³⁻⁵ on the subject in *Nature*. On May 15, 1970, at a point some 80 to 150 km east of St Louis, an aircraft in which I was a passenger was forced to fly into and through a thick dark storm-cloud mass. Extreme turbulence resulted in a rather violent movement of the wings of the aircraft (a Boeing 727). All this time the aircraft was descending, and an electrical display was lighting the surrounding clouds in a diffuse glow. The frequency of electrical discharges (no actual strokes were seen) increased with the increasing turbulence. At (what seemed) the moment of maximum turbulence and electric discharge, while the aircraft was still descending through the storm, a sequence of events took place that I list below. The whole took not more than 5 s, and I must admit that the order, save for numbers 4 and 6, is not necessarily chronological. 1, The turbulence ceased altogether. 2, The surrounding electrical discharges (glows) ceased altogether. 3, The wing stopped buckling altogether. 4, A white glowing sphere (ball lightning?) appeared on the port wing tip. I do not know if it was actually touching

the wing. Its diameter was less than 1 m and more than 10 cm. Its boundary was "fuzzy" and not distinct. 5, There was a soft "pop". 6, The ball lightning (?) vanished.

Regarding the accompanying noise (5) I recorded it at the time. Shortly afterwards my scientific upbringing and I both decided that an outside noise was not likely to be heard within a moving jet aircraft, and that my eye, seeing the ball go, insisted to my ear that it should do so accompanied by a noise. Nonetheless, I record it now, as I recorded it then.

Yours faithfully,

MURRAY FELSHER

Council on Education in the Geological Sciences,
American Geological Institute,
2201 M Street, NW, Washington DC.

¹ Powell, J., and Finkelstein, D., *Amer. Sci.*, **58**, 262 (1970).

² Altschuler, M. D., in *Scientific Study of Unidentified Flying Objects*, sect. VI, chap. 7, London, E. V., Director, USAF Contr. F44620-67-C-0035 (1969).

³ Covington, A. E., *Nature*, **226**, 252 (1970).

⁴ Lillienfeld, P., *Nature*, **226**, 253 (1970).

⁵ Bromley, K. A., *Nature*, **226**, 253 (1970).

Obituaries

Professor S. Chapman

SYDNEY CHAPMAN, FRS, who died on June 16, made fundamental contributions to many subjects, especially gas theory, geomagnetism and atmospheric physics.

While still a Cambridge student, at Larmor's suggestion he began to work on the problem of viscosity, heat conduction and diffusion in a gas. This problem, posed by Maxwell and Boltzmann forty years earlier, had been solved only for two special types of molecular interaction; Boltzmann had stated that one must almost despair of a general solution. This was true if one sought an exact solution. An approximate solution, correct to any appropriate degree of accuracy, was, however, possible, and Chapman in 1915-17 provided such a solution, based on an equation of transfer given by Maxwell. Shortly afterwards (1917) Enskog independently gave a solution, based on the theory of integral equations. This, though more elegant in formulation, was essentially equivalent to Chapman's; thus one normally speaks of the Chapman-Enskog theory.

Chapman was especially concerned with applications of the theory to actual gases. The theory predicted two new phenomena, gaseous thermal diffusion and a converse thermo-diffusion effect. Chapman was delighted when Dootson in 1917 detected thermal diffusion and when Waldmann later demonstrated the converse effect; still more when Clusius in 1939, by his separation column, realized a dream of Chapman's, dating from 1919, that thermal diffusion might be used to separate isotopes. In 1922 Chapman was applying his results to plasmas (especially in stars); his work on the upper atmosphere, from 1925 on, relied on a background of kinetic theory. After the publication in 1939 of *The Mathematical Theory of Non-Uniform Gases*, written jointly with Cowling, he concentrated less on kinetic theory problems, though he reverted to them as new ideas struck him. It was fitting that the third edition of the book appeared just a month before his death.

When he left Cambridge, Chapman was appointed senior assistant at the Greenwich Observatory. He left Greenwich after only three or four years to devote himself to other interests; nevertheless, those few years in large measure determined his later research. At Greenwich he began by supervising the reconstruction of the magnetic

observatory. He found that magneticians were far readier to collect than to interpret data, and set himself to analyse geomagnetic variations. A visit from Schuster, a pioneer in the theory of geomagnetic variations caused by tides in the ionosphere, stimulated him to work both on the dynamo theory of the solar and lunar variations, and on tides in the upper atmosphere. Dyson drew his attention to the apparent connexion between solar flares and magnetic storms, so sparking off yet another series of investigations.

Chapman's research followed several lines, each systematically pursued over a number of years; sometimes he was working along several different lines at once. His work on the analysis of geomagnetic variations covered chiefly the years 1914-31; for it he was awarded the Adams Prize for 1927-28, and the work was later expanded into the two-volume epic *Geomagnetism* (1940), written jointly with Bartels. His main work on lunar atmospheric tides was spread over the years 1918-48. Before it, such tides could be securely inferred from barometric records only near the equator. Chapman isolated the tide from Greenwich records, and went on to determine its properties over the globe; to do so he had (especially with Miller) to devise methods of data processing at a time when mechanical aids were limited. This and the work of others was expounded in the recent book, *Atmospheric Tides* (1970), written jointly with Lindzen.

Chapman's work on magnetic storms began in 1919 with a paper, the theoretical part of which he later acknowledged to be invalid. During the next ten years he cautiously surveyed various aspects of the problem, but not until 1931 did he, in company with Ferraro, reach the idea that magnetic storms occur because streams of solar particles compress the geomagnetic field. This idea, predating observations of the magnetospheric cavity by thirty years, explained only the initial phase of a storm; not until after the discovery of the van Allen radiation belts could it be completed. Chapman shared with others in its completion, this time working with Akasofu (1961). He also introduced Akasofu to work on aurorae, which had long fascinated him, and took a lively interest in the solar wind, the continuous flow of which he had, in 1955, narrowly missed predicting.

In his Bakerian lecture (1931) Chapman gave the theory

of an ideal ionospheric layer, since named after him. He has also discussed the formation of ozone, other photochemical atmospheric problems, diffusion in the atmosphere and recently (with Kendall) noctilucent clouds. Fittingly, it was he who introduced the word 'aeronomy' for the science of the upper atmosphere.

He served as president for the Royal Astronomical Society and the International Union of Geodesy and

Geophysics, among other organizations. He was also chosen to act as president of CSAGI, the special committee for organizing the International Geophysical Year, a venture which he strongly supported and which owed much to his efforts. Officially he retired from his Oxford chair in 1953; in practice the effect was to double his activity and extend it over the world. His quiet, friendly, committed and wise counsel will be greatly missed.

Announcements

University News

Professor R. J. Kitz, chief of anaesthesia at the Massachusetts General Hospital, has been appointed Henry Isaiah Dorr professor of research and teaching in anaesthetics and anaesthesia at **Harvard University**, in succession to **Professor Henry K. Beecher** who will become Henry Isaiah Dorr professor emeritus. **Mr C. Richard Taylor**, of Duke University, has been appointed first director of the **Concord Field Station of Harvard's Museum of Comparative Zoology**.

The following appointments have been made in the **University of London**: **Mr J. N. Britton**, to the Goldsmith's chair of education tenable at the Institute of Education and attached to Goldsmiths' College; **Professor B. R. Rabin**, to the chair of biochemistry, University College; **Dr G. F. M. Russell**, to the chair of psychiatry tenable at the Royal Free Hospital School of Medicine; **Mr G. N. Walton**, to the chair of nuclear technology at the Imperial College of Science and Technology; **Dr D. V. Bugg**, to the chair of nuclear physics, Queen Mary College; **Professor S. J. G. Semple**, to the chair of medicine tenable at the Middlesex Hospital Medical School. The title of professor of entomology has been conferred on **Dr Reginald F. Chapman**, in respect of his post at Birkbeck College, that of professor of rock mechanics has been conferred on **Dr Evert Hoek** in respect of his post at the Imperial College of Science and Technology, and that of professor of chemistry has been conferred on **Dr A. J. B. Robertson**, in respect of his post at King's College. The title of Emeritus professor has been conferred on the following: **Professor E. Boyland** (biochemistry, Institute of Cancer Research); **Professor F. Brailsford** (electrical engineering, University College); **Professor F. C. Champion** (experimental physics, King's College); **Professor F. E. Camps** (forensic medicine, London Hospital Medical College); **Professor G. W. Causey** (anatomy, Institute of Basic Medical Sciences); **Professor S. J. De Navasquez** (pathology, Guy's Hospital Medical School); **Professor S. E. Dicker** (physiology, Chelsea College of Science and Technology); **Professor F. Goldby** (anatomy, St. Mary's Hospital Medical School); **Professor J. Greig** (electrical engineering, King's College); **Professor W. J. Hamilton** (anatomy, Charing Cross Hospital Medical School); **Professor H. R. Hewer** (zoology, Imperial College of Science and Technology); **Professor A. Kekwick** (medicine, Middlesex Hospital Medical School); **Professor N. F. MacLagan** (chemical pathology, Westminster Medical School); **Professor Sir John Randall** (biophysics, King's College); **Professor J. W. Smith** (chemistry, Bedford College); **Professor R. S. Stacey** (pharmacology and chemotherapeutics, St Thomas's Hospital Medical School); **Professor Lord Stamp** (bacteriology, Royal Postgraduate Medical School); **Professor J. S. Wilkie** (history and philosophy of science, University College).

Appointments

Dr A. F. Spilhaus has been appointed executive director of the **American Geophysical Union** in succession to **Dr Waldo E. Smith**, who has retired after 25 years of service with the organization.

Professor George W. Beadle, University of Chicago, and **Mr Thomas H. Coulter**, Chicago Association of Commerce and Industry, have been appointed co-chairmen for the 1970 meeting of the **American Association for the Advancement of Science**.

The first three senior appointments to the new **Medicines Inspectorate** have been made: **Mr A. G. Fishburn** is to be principal medicines inspector and **Mr R. Baker** and **Mr W. P. Jones** will be senior medicines inspectors. Mr Fishburn was formerly with the ICI Pharmaceuticals Division, and Mr Baker and Mr Jones with Parke Davis and Cyanamid, respectively.

Miscellaneous

To mark **Professor H. R. Hewer's** golden jubilee and retirement from the chair of zoology at Imperial College, a dinner will be held at the college on October 1, 1970. A presentation will be made at a sherry party before the dinner. If any of Professor Hewer's friends or students have not received details of the celebration, they should contact Dr D. M. Kermack, Zoology Department, Imperial College, Prince Consort Road, London SW7.

Sabbatical Itinerants

From the issue of September 5, entries of this kind will appear among the classified advertisements and will be charged for accordingly. Copy should be addressed to T. G. Scott and Son Limited, 1 Clement's Inn, London WC2A 2ED

Exchange: Cork (Ireland) architect taking one year postgraduate course at the University of Edinburgh wishes to exchange furnished house in Cork in own grounds of 2 acres, 3 miles from city centre and university, for house in or near Edinburgh from October 1, 1970, to June 30, 1971. Please contact G. F. McCarthy, Oldwood, Rochestown, Co. Cork, Ireland.

Wanted: Comfortable 4 bedroomed home in Oxford area from March 25, 1971, to August or September 1971, for considerate family with 4 children, ages 15-20. 5 bedroomed home available in Seattle. Please contact Professor B. S. Rabinovitch, Department of Chemistry, University of Washington, Seattle, Washington 98105, USA.

British Diary

Wednesday, September 2

132nd Annual Meeting (eight days) British Association for the Advancement of Science, at Durham.

Man/Computer Interaction (three-day conference) Institution of Electrical Engineers, and the Institution of Electronic and Radio Engineers, at the National Physical Laboratory, Teddington, Middlesex.

Sunday, September 6

RF Electrical Measurement Practice (thirteen-day vacation school) Institution of Electrical Engineers, and the Institution of Electronic and Radio Engineers, at the University of Kent, Canterbury.

Monday, September 7

Basic Concepts in Modern Control Theory (five-day vacation school) Institution of Electrical Engineers, at the University of Birmingham.

Control Mechanisms of Growth and Differentiation (five-day symposium) Society for Experimental Biology, in conjunction with the Society for Developmental Biology, at Eliot College, University of Kent, Canterbury, and Wye College (University of London), Wye, Ashford, Kent.

Computational Physics (three-day conference) Institute of Physics and the Physical Society, at Imperial College, London SW7.

Third International Broadcasting Convention (five days) Institution of Electrical Engineers, and the Institution of Electronic and Radio Engineers, at Grosvenor House, London W1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

- Music and the BBC. By Charles Curran. Pp. 12. (London: BBC, 1970.) [277]
 Cement and Concrete Association. Report for the year 1969. Pp. 66. (London: Cement and Concrete Association, 1970.) [277]
 Overseas Development Institute. ODI Review—4: British Development Policies, Needs and Prospects, 1970. Pp. 96. (London: Overseas Development Institute, Ltd., 1970.) 20s. [277]
 Anti-Locust Research Centre. Anti-Locust Bulletin No. 47: Environmental Control of Coloration in an Acridid, *Gastrimargus africanus* (Saussure). By C. H. Fraser Rowell. Pp. 48+1 plate. 19s (95p). No. 48: The Development of Eggs of the Red Locust *Nomadacris septemfasciata* (Serv.), and the African Migratory Locust *Locusta migratoria migratorioides* (R. and F.), and its Interruption Under Particular Conditions of Humidity. By A. S. Shulov. Pp. 22. 10s (50p). (London: Anti-Locust Research Centre, 1970.) [277]
 Chelsea College, University of London. Prospectus, 1971/1972. Pp. 90. (London: Chelsea College, 1970.) [277]
 The Scottish Horticultural Research Institute. 16th Annual Report for the year 1969. Pp. 70. (Invergowrie, by Dundee: Scottish Horticultural Research Institute, 1970.) [277]
 Ministry of Housing and Local Government. Welsh Office. Taken for Granted—Report of the Working Party on Sewage Disposal. Pp. vii+65+5 plates. (London: HMSO, 1970.) 10s (50p) net. [286]
 Comments on Earth Sciences: Geophysics, Vol. 1, No. 1, June–July, 1970. Pp. 1–27. Subscription rates. Published bi-monthly. Each volume consists of six issues. In Great Britain: Individuals who warrant the journal is for their own personal use, per volume, post paid, 83s 6d. Libraries, research institutions and others, per volume, postpaid, 175s. Outside Great Britain: Individuals who warrant the journal is for their own personal use, per volume, post paid \$11, 91s 6d. Libraries, research institutions and others, per volume, post paid, \$25, 208s 6d. (London and New York: Gordon and Breach, Science Publishers, 1970.) [297]
 Ministry of Technology. Working Group on Engineering Training and the Requirements of Industry. Graduate Training in Manufacturing Technology. Pp. vi+30. (London: HMSO, 1970.) 4s 6d (22½p) net. [297]
 University of Oxford. Annual Report of the Curators of the Bodleian Library for 1968/1969. (Supplement No. 9 to the *University Gazette*, Vol. C, July, 1970.) Pp. 59. (Oxford: The University, 1970.) 8s. [297]

- Queen Mary College, University of London. Annual Report, Session 1968/1969. Pp. 143. (London: Queen Mary College, 1970.) [297]
 Science Research Council. Greenwich Time Report. Time and Latitude Service, 1969 July–September. Pp. 95–106. (London: Science Research Council, 1970.) [307]
 Test Sieve Data. Pp. 96. (London: Endecotts (Filters), Ltd., 1970.) [317]
 John Innes Institute. Sixtieth Annual Report. Pp. 69. (Norwich: John Innes Institute, 1970.) 30p. [317]
 Commonwealth Mycological Institute and Association of Applied Biologists. Descriptions of Plant Viruses, Sheets 1–20, Set 1. (Kew: Commonwealth Mycological Institute, 1970.) 30s; \$3.90. [317]
 The Soil Survey: England and Wales—Annual Report for 1969. Pp. 34. (Harpenden: Soil Survey of England and Wales, Rothamsted Experimental Station, 1970.) [317]
 Home-Grown Cereals Authority. Progress Reports on Research and Development, 1969/1970. Pp. 43. (London: Home-Grown Cereals Authority, 1970.) [388]
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 Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 258, No. 827 (3) July 1970: The Fine Structure of the Vertical Lobe of Octopus Brain. By E. G. Gray. Pp. 379–394+plates 73–92. (London: The Royal Society, 1970.) 40s; \$5.20. [388]
 British Antarctic Survey. Scientific Reports, No. 57: The Geology of Adelaide Island. By G. J. Dewar. Pp. 66+7 plates. (London: British Antarctic Survey, 1970.) 55s net. [484]
 A Readers' Guide to Britain and the European Communities. By Carol Ann Cosgrove. Pp. 106. (London: Chatham House and PEP, 1970.) 15s. [484]
 London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, Management Studies and Commerce, 1970/71. Part 1: Autumn Term. Pp. 112. (London: London and Home Counties Regional Advisory Council for Technological Education, 1970.) 10s. [58]
 The Radiochemical Centre. Medical Monograph No. 7: Determination of Steroid Hormones. By Ian F. Sommerville. Pp. 48. (Amersham, Bucks: The Radiochemical Centre, 1970.) [68]
 Building Research Station. Current Paper 19/70: Discomfort in Schools from Overheating in Summer. By F. J. Langdon and A. G. Loudon. Pp. 10. (Garston, Watford: Building Research Station, 1970.) *Gratis*. [68]

Other Countries

- Science Council of Canada. Annual Report 1969/1970. Pp. 42. (Ottawa: Queen's Printer, 1970.) [307]
 Search: Science, Technology and Society, Vol. 1, No. 1, July, 1970. Edited by J. B. Davenport. Pp. 1–48. (Sydney: Australian and New Zealand Association for the Advancement of Science, 1970.) [307]
 Jamaica: Ministry of Agriculture and Fisheries. Commodity Bulletin No. 3: Pimento: a Short Economic History. By D. W. Rodriguez. Pp. 52. (Kingston, Jamaica: Ministry of Agriculture and Fisheries, 1970.) [38]
 Moving the Obelisks: a Chapter in Engineering History in which the Vatican Obelisk in Rome in 1586 was Moved by Muscle Power, and a Study of More Recent Similar Moves. By Bern Dibner. Pp. 61. (Cambridge, Mass. and London: The M.I.T. Press, 1970.) \$2.95. [38]
 The Floods of Southeastern Europe, May–June 1970. Pp. 7. (Washington, DC: Smithsonian Institution, Centre for Short-Lived Phenomena, 1970.) [38]
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 Australia: Commonwealth Scientific and Industrial Research Organization, Land Research Series No. 26: Lands of the Mitchell–Normanby Area, Queensland. Comprising papers by R. W. Galloway, R. H. Gunn and B. Story. Pp. 101+2 maps. (Melbourne: CSIRO, 1970.) [68]

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Is there Time for Thinking ?

IN characteristically different ways, Lord Todd, President of the British Association this year, and Professor John Ziman have raised at the British Association questions about the institutional structure within which science must be carried on in countries such as Britain. Lord Todd is concerned (see page 988) to argue that there is a need to match the educational system more sensitively to the needs of professional scientists and to the frequently conflicting needs of the young people entering into higher education. Professor Ziman is rightly alarmed that the character of scientific work may be damaged by some of the institutional forms which have emerged or which may emerge, but at the same time anxious that people should more explicitly acknowledge the importance of the conventions which keep professional behaviour within seemingly bounds and which have given science its reputation for integrity. One of the oddest features of the development of science in the past few years has been the tendency to take these institutional forms for granted. The title of Lord Todd's address—"A Time to Think"—is intended as a plea that the time has come for a reorganization of the educational reappraisal of what society can expect of scientific work, and nobody will quarrel with him by denying the need for change. The question is whether there is time enough.

The defects of the educational system—not merely in Britain but wherever universities offer science as a part of their curriculum—have been plain for donkeys' years, and one of the pathological signs that Professor Ziman might have included in his catalogue is the shameful way in which academics have taken these educational problems seriously only since what is called the drift away from science made them alarmed that the need for university teachers of science might one day disappear. Even when students were fighting to get into the laboratories, however, it was plain that the structural defects of the undergraduate curriculum had become an impediment to education in the broader sense. It is a great source of shame that so little should have been done, in the past few years, to put this right.

What needs to be attended to? First of all, there is the important question of entry requirements to the universities. Wherever dons are gathered, it is comparatively easy nowadays to win agreement that students and universities would be better off if secondary education (in Britain) were more liberal. Even chemists will sometimes be ready to admit that their students would be able to survive the rigours of university education in chemistry without first having been through exactly the same hoops at school.

Moreover, there has in the past few years been a kind of understanding between the universities and the schools that there should be negotiated the basis for a broader sixth-form education—the common assumption is that when the protracted negotiations between the Schools Council and the Standing Conference on University Admissions are eventually completed, sixth-formers will be expected to prepare themselves in five subjects, not simply three as at present. This is a meagre reform for which to wait so long. And there is very little hope that the new pattern of school studies and university entrance requirements will carry the system forward to the point at which a student's work at university or elsewhere in the higher educational system may be quite unrelated to his work at school. The result will be not merely needless specialization at schools and the loss of potentially bright people to potentially rewarding careers by the accidents of their arbitrary choices in extreme youth, but also a reinforcement of those pressures, already far too powerful, which isolate one part of the educational system from another, thus diminishing the power of the academic world to stimulate itself. It goes without saying that the same requirement that higher education should be open only to those who by demonstration in advance are least in need of it perpetuates the educational privilege of the better schools. It is a scandal that the universities as a whole and university teachers of science in particular should have been as complacent as in the past few years in tolerating this state of affairs.

The internal defects of higher education have similarly been neglected for too long. Again it is something of a scandal in Professor Ziman's delicate sense that interest in reform has been awakened largely by external pressures. In Britain, for example, the Committee of Vice-Chancellors seems to have given lukewarm support to the notion of a two-year degree course chiefly because this seems to offer some relief from the continually mounting pressures of the demand for more and more higher education. With encouragement from the government (anxious to save a little money as well as to make the system grow), the Vice-Chancellors gave timid support to the concept of a two-year university course as a preparation either for vocational training of some kind or as a preparation for more advanced scholarship. But the device which now seems desirable on the grounds of expediency has been educationally sensible for a long time. Professor Brian Pippard, for example, made an eloquent argument in favour of such a scheme some time ago (*Nature*, **219**, 1307; 1968). The way things have turned out in Britain, there is good reason to believe that the usual

three-year course for undergraduates contains too much teaching for many people but that it is certainly too skimpy as a preparation for a career in science as such. Educational arguments are sufficient to force this conclusion.

Important questions about the character of university education in science are also habitually overlooked. Needless specialization is rife. Chemists, for example, are taught thermodynamics as if it were a discipline quite distinct from that drummed into physicists. At many universities, it is still possible for an undergraduate to be taught biology well, while remaining innocent of the mathematical techniques which have already been tools of the trade. And in many universities, attempts are being made to give courses what their designers fancy will be greater relevance by throwing in a few bits and pieces from courses in economics or management. Altogether, there is too little care for embodying in university courses the qualities of an intellectual kind that make scientific work as such intellectually exciting. In many ways, it is a surprise that university courses in science have retained some attractiveness for young people when so little has been done to elevate them above the status of compendia of manipulative techniques.

The same is true of higher degree courses at the universities. In the past few years, research has been made almost a mockery of what it might be by the mindless growth of many graduate schools. Professor Ziman points out that the PhD process has become too automatic. The Western world abounds with university departments in which an important piece of equipment, an NMR machine perhaps or a mass spectrometer, becomes the means by which dozens of young men and women go through the motions of research, fill out their theses and, in due course, are moved one rung up the ladder. Because snobbishness is such an easy trap in discussions of this kind, it is important explicitly to acknowledge that the old-fashioned PhD system had such serious flaws that it is probably now unworkable. It is too much to ask that every candidate for a higher degree should carry out some entirely original piece of research, more or less unaided by his superiors. Indeed, it is important now to recognize that the higher degrees are means by which people are trained in the techniques of research and are at the same time provided with opportunities—which many people seize—to become scholars in the old-fashioned sense as well. Among educationists, it should now be a burning question to know how these occasionally conflicting needs should be satisfied in modern universities. The chances are that any pattern of advanced work that seemed more appropriate than the present would be one in which there were more incentives for young people to make original contributions to science outside the framework of what has become the conventional pattern of success. Is it too much to ask that a man's achievement should be marked by the influence which his work has on other people's work, and not by the flimsy signs of that influence consisting of the appearance of scientific

papers in the journals and by the award of appropriate degrees?

The literature is a splendid embodiment of Professor Ziman's little pathologies. Among scientists, it tends to be assumed that the scientific literature exists for the benefit of authors, not readers. The result is that much of the literature is unread except by the growing army of abstractors, cataloguers and the like. Those who contribute to the published literature are sometimes simply concerned with establishing priority for a discovery, the significance of which it is too soon to assess. Often, the obscurity of a paper can be traced directly to the inadequacy of its preparation; yet is it not an offence against the doctrine that the scientific literature should be the living embodiment of the accumulation of discovery that some of it should, with the knowledge and consent of its authors, be too obscure to be understood? Professor Ziman might have added to his list of the little weaknesses of professional science the temptation for people to hug their discoveries to themselves—Hooke and his anagrams are a good illustration. But is there not in the defects of the scientific literature in modern times a powerful streak of this unwillingness to be fully understood? And is that not at least a partial denial of the nature of intellectual life, in which deeper understanding usually springs from attempts by honest people to understand what other people have to say?

Professor Ziman is right, of course, to emphasize that to make too much of these defects would be to underestimate the essential health and integrity of the profession of science. That is true. Too much crying of stinking fish may indeed provide unwarrantable excuses for mindless criticism of the profession of science. There is, however, a real dilemma to be resolved. If, for the sake of not giving hostages to fortune, too little is said about the structural defects within science, there will be no hope that they will ever be repaired. This is why even such apparently unseemly matters as the bumbledom of the national academies, or the arbitrariness of the system for awarding Nobel Prizes, need to be talked about in public if the institutions concerned are not themselves to be overwhelmed by the reputation for anachronism. But criticism, however constructive, cannot by itself be a way of mending what is wrong. The more urgent need is that there should be a recognition among practising scientists that the scientific life is an intellectual life, not merely an extension into the universities of modern business. To admit this does not in any sense imply that science has nothing to contribute towards prosperity and public health—most probably the shoe is on the other foot, for a more vigorously intellectual profession of science would be an even more prolific source of innovation. Certainly it could hope to become again the focus for the intellectual idealism of the young people who used to throng into the laboratories. The most serious danger now is that with the mounting pressures from outside—the demands for economics and for relevance, whatever that may be—there may be no time left for thinking.

Putting Power Stations where they Belong

THE row about the siting of a new electricity generating station for New York, settled by one of Mr John Lindsay's characteristic compromises (see page 992), is more than a local squabble or even a special feature of what remains the most remarkable of all cities. In one way or another, most modern cities find themselves hard pressed to know just how to keep themselves supplied with electricity. In many places—London is all too obvious an example—the solution has been to build generating plants as close as possible to the points at which the electricity is consumed. The result has been either disfigurement or needless air pollution or a combination of both. It follows that there should be a deliberate attempt to isolate the lessons that can be learned from the maladroitness for the building of the Astoria power plant in New York, for even if the mayor has for the time being made sure that the Astoria plant will be the last in New York to burn fossil fuel, his writ does not run elsewhere.

The lack of forward planning is perhaps the most obvious and the most serious defect of the scheme which is now to be half implemented, and it is only fair to say that the utility company, Consolidated Edison, is not entirely to blame. For several years the company has been hindered in its schemes for building power stations for New York by considerations for the quality of the environment. In the early sixties, for example, the company was prevented from building a nuclear power station more or less across the river from the United Nations headquarters and even if, now as then, the company seems to have been foolishly willing to mix technology and people's living spaces, its arguments about the urgent need of generating capacity have been plain enough. The prospect earlier this summer that there would not be enough generating capacity to keep the air conditioning sets in operation, which happily has not materialized, has been a powerful reminder that cities are unworkable without electricity, and that it is necessary to plan for future growth three or even five years ahead. So much everybody will concede.

But is it necessary to meet the growth of a city's need of electricity by building power stations on the spot? This is the most constructive of the questions raised in the protest about the Astoria power station in the past few weeks. The answer, of course, is that there is no technical reason why a city even as large as New York should not be supplied with electricity generated at a remote site. To be sure, the cost of transporting electricity over distances of the order of some hundreds of miles is not negligible, especially when the alternatives are power station sites that can be supplied cheaply with fossil fuels by sea or river; yet the cost is at the same time not outrageous. In the United States, where the interconnexion of the electricity generating systems is not nearly as well developed as in Europe and where

the economic advantages of interlinked capacity have not been exploited to the full, it would be surprising if the real extra cost of building power stations away from cities were as much as an extra 10 per cent. The way in which many utility companies plan to make money by generating electricity at remote nuclear installations is a sufficient proof that distance need not be an insuperable obstacle. In this sense, Mr Lindsay is entirely right to have insisted, in his agreement with Consolidated Edison, that the local utility should develop its distant connexions. That is a step that other city authorities could usefully take.

It is entirely within the bounds of possibility that the demand for electricity in a city such as New York could be substantially reduced by proper design. The question has received no attention worth speaking of, but the point has probably now been reached at which buildings might be designed to be kept comparatively cool in summer without making such a large demand on the air conditioning supplies. Instead of throwing up their hands in horror at the condition of the modern city, people should pay some attention to problems like these.

100 Years Ago



NOTES

A WEEK or two ago we announced a rumour to the effect that the Government had refused to allow a ship to convey the eclipse observers to Spain and Sicily next December. The rumour was too well founded; the Government has actually refused to tell off a ship for this purpose. This decision in the teeth of the plainest precedents requires no comment on our part; in fact, it is beyond all comment, it is astounding. We are enabled to announce, however, that the American Government, more enlightened than our own, are making extensive preparations; and upon the results of their labours and those of the Continental Governments Englishmen must therefore fall back, in a research which is eminently English. The Americans will send three corps of observation, to be stationed respectively at Malaga, Sicily, and some place in Turkey most available for making the best scientific records and views. One of these corps will be sent from the Naval Observatory, and the other two will be composed of the most scientific men in the country, including the professors from Harvard University. Before the war broke out it was arranged that Rear-Admiral Glisson should extend to the corps at Sicily all the aid and co-operation in his power. But the original plan has been spoiled for the present by the troubles in Europe, Admiral Glisson being obliged to move his squadron to the Baltic for the protection of American commerce in that vicinity.

From Nature, 2, 379, September 8, 1870.

OLD WORLD

BRITISH ASSOCIATION

Thinking about Education

THE British Association for the Advancement of Science opened in Durham last Wednesday with an address by the association's president, Lord Todd of Trumpington. The burden of Lord Todd's message is that "while science and technology have been bringing about vast changes in our material existence at an ever-increasing pace we have failed to match them with appropriate social and educational changes". The symptoms of this failure are "the frustration evident among young people" and a disillusionment with science which is sharpened by concern about pollution and deterioration of the natural environment.

Such a theme, as Lord Todd himself points out, is hardly new, but his chief concern is that appropriate steps should be taken to remedy the situation, and that is why the theme bears repeated discussion from all angles. This, according to Lord Todd, "is a time to think", especially about the structure and content of the education system.

Education holds the central position in Lord Todd's thinking for a variety of reasons, but mainly because educational patterns and social attitudes go hand in hand. Until school and university curricula are taken out of the straitjacket of specialization, and until natural science is recognized "as much a branch of culture as music, literature and the arts and not in any sense a subject for the specialist alone", it will always be viewed with scepticism. There will therefore be little hope of creating a scientifically conscious democracy capable of taking rational decisions about science policy until society, through education, is made aware of the nature of science.

The place to start, according to Lord Todd, is in the secondary schools, by broadening, curricula and putting an end to early specialization. But this will have little effect as long as the virtues of traditional university education are emphasized at the expense of other types of tertiary education. One of the chief malaises of British education in Lord Todd's view is that too many students are clamouring at the doors of the universities for an education to which many of them are unsuited. It is therefore not only unrealistic but also positively damaging to suppose that the official estimates that 450,000 students will be accommodated in British universities by 1980 mean that many more students will be receiving the benefits of university education. "What a large proportion of that 450,000 ought to pursue is some other form of higher education with a different and greater vocational bias."

The trouble with regarding university education as the ultimate academic aim for which every school-child should strive, according to Lord Todd, is that university graduates ultimately end up in jobs for which their training is completely unsuitable, and they become frustrated. This frustrated white-collar class is "in part responsible for the so-called 'drift from science'". Lord Todd therefore believes that urgent thinking is required, before the debate about the future of higher education draws to a close.

COUNTRYSIDE

Boost for Linear Planning

by our Planning Correspondent

FROM the date of its nationalization (January 1, 1948) to December 31, 1968, British Railways closed 5,618 route miles (9,041 km) of railways in England and Wales. In other words, as the Countryside Commission so vividly describes in a new report, the area of railway land which has been falling into disuse each year is roughly equivalent to the land requirements of a new town of 80,000 persons (*Disused Railways in the Countryside of England and Wales*, HMSO; £1). Much of this railway land remains derelict, and only about a third of the mileage on the market between 1948-68 was actually sold (see Table 1). Obviously in a country as crowded as Britain, such land ought to be put to use again, but the disposal of disused railway lines seems to be fraught with difficulties according to Dr J. H. Appleton, who prepared the report for the Countryside Commission.

Table 1. RECENT RATES OF CLOSURE AND SALE OF RAILWAY IN ENGLAND AND WALES

	Length closed (km)	Length sold (km)	Excess of length closed over length sold (km)
1965	1,002	204	798
1966	1,209	449	761
1967	568	488	80
1968	641	528	113
1965-68	3,420	1,669	1,752

Part of the problem is that the use of railway land is limited to a large extent by its physical characteristics—its linear form, gradient, curvature and the like—and its sometimes complicated relationship with the adjacent land. A further difficulty is that, at present, while the government requires the British Railways Board to offer the land first to local planning authorities, the authorities usually decline to buy it and British Rail then offers it elsewhere to the highest bidder with the result that land is often sold piecemeal. The whole procedure, according to Dr Appleton, is complicated, often protracted, rarely integrated, and hardly in the public interest as a whole. But this land-use problem is not one, Dr Appleton says, that can be solved by a blanket decision that converts all disused railway lines into agricultural land, long-distance footpaths, bridleways or roads, however worthy these potential uses. Each stretch of railway land needs to be judged on its own merits and costed efficiently, although its disposal must also be seen in the wider context of a general land-use policy; for example, some lines might be integrated into an existing reclamation scheme.

Dr Appleton suggests therefore that the disposal of disused railway land should be taken out of the hands of British Railways and passed to some new body which could look at the matter in a wider context. Alternatively, the local planning authorities could be responsible, or even the Countryside Commission which, given more staff, could coordinate policy. Although there will continue to be conflicts of interest (particularly between agricultural and recreational uses), Dr Appleton believes they would be reduced if decisions were logical, if the public were involved, and also if they were consistent with declared land-use policy.

DEFENCE

A Hole in Skynet

from a Correspondent

AN inquiry is now going on at Cape Kennedy as to the cause of the disappearance of the Skynet 1 defence communications standby satellite built under contract for the British Ministry of Defence by Philco-Ford. The successful launch by a US Air Force Delta rocket took place on August 19, but a week later when the satellite was due to be "kicked" into its 30,000 kilometre geostationary orbit above the Indian Ocean, telemetry signals failed during firing of the apogee motor (which performs the final orbital manoeuvre). The result is that the satellite may well be in its correct orbital position without anyone being the wiser. No contact has been obtained since, although hopes of tracing the satellite (presumably by radar) have not entirely faded. If traced, it is possible that the satellite could still perform its purpose—acting as a high-speed voice link for the armed services between England and the Far East if its predecessor, launched last year, ever becomes out of action. At present, the first Skynet 1 satellite is still fully active and there is a contractual bonus for each additional year in which it keeps working, up to 5 years.

Specifications for a revised, second generation satellite link for the Ministry of Defence, called Skynet 2, have already been drawn up, this time with British contractors in view. The first replacement by Skynet 2 is called for in 1973. Hawker-Siddeley Dynamics and GEC are preparing preliminary design studies, one of which is expected to be chosen later this month.

Various questions now arise. If the second Skynet 1 proves irretrievably lost, will the Ministry of Defence be able to soldier on for up to three years without a standby until Skynet 2 is ready? (It has managed without for nine months so far.) Is any compensation payable in the form perhaps of a free replacement launch by the Americans? An apogee motor is integral with the satellite, though it may be considered part of the launch. Is western security considered at risk? Upon this, as much as upon the results of the current inquiry, may depend the terms on which a further Skynet 1 is, or is not, speedily in orbit.

BRITISH SPACE

Dead-end Rocket

THIS week Britain has been shamefacedly and unsuccessfully trying to enter space with a rocket for which there is no future. Even the space hawks have been showing embarrassment, not joy. A satellite could have been launched by a British rocket as much as ten years ago, they were saying before the firing. Seen from Britain, the preparations of the Black Arrow rocket on the launch pad at Woomera seemed more like a wake than a step through a new frontier, and the delays, first of all because of a fault in the tracking station at Gove, northern Australia, only prolonged the agony.

Black Arrow's genealogy begins with the Black Knight research rocket that was first fired twelve years ago, with an injection of engine technology from the Blue Steel stand-off bomb. On average one Black Arrow rocket is being made each year in a programme that is costing about £3 million per year but is expected

to rise to about £5 million during the next five years. The cost of this week's rocket and its payload is believed to be in the region of £1 million. This expenditure, the Ministry of Technology would say, will buy prestige that will help the electronics industry, even though satellites have already been launched by five other countries including the arch-competitor, Japan. Later Black Arrow launchings of technological satellites to test new solar cells and the like, Mintech says, "will represent a major step in the development of application satellites". Yet it is inconceivable that Black Arrow could ever launch a satellite into a geostationary orbit, and this is where communications satellites have to be. Even with four Skylark rockets strapped to the first stage, a scheme being considered to double the payload to about 400 lb., Black Arrow could not carry a geostationary satellite and the additional motor to get it there.

What then will Black Arrow achieve? At best it has kept Britain's hand in, an insurance policy to mature when British space policy extricates itself from the two stools between which it has fallen. Either Britain will have to go in with the Europeans, in a more full-blooded scheme than has existed so far, to exploit the technology that is available for scientific and commercial use, or remain at the present level, launching satellites that belong to the first decade of space research. Space technology has gone beyond the Black Arrow stage to heavy communications satellites and the observatory type of scientific satellite.

URANIUM

How Much Ore in Scotland?

IT would be premature at this stage to see the makings of a new "uranium rush" in the disclosure last week that deposits of uranium ore in northern Scotland are substantially more widespread than had hitherto been considered. Although a survey for uranium, being carried out for the Atomic Energy Authority by the Institute for Geological Sciences, has shown indications of significant quantities of uranium minerals around Ousdale, Helmsdale and Brawlbin in Caithness there is still no hint of commercially viable deposits on the evidence so far.

In one area near Ousdale, however, a zone of something over a kilometre in length was located containing quantities of uranium ore. Anomalies in surface and below-surface radioactivity were detected in several other neighbouring areas and in their first report of the findings Dr S. H. U. Bowie, Mr D. Ostle and Dr M. J. Gallagher point out that there is certainly enough evidence to warrant further investigation of these areas.

The Atomic Energy Authority are looking to establish mining interests to follow up this challenge. Although the British nuclear power industry spends at present only about £7.5 million a year on uranium concentrate this figure is increasing all the time, and the AEA are obviously conscious of the long-term saving which a substantial uranium find would offer.

The survey reported by Dr Bowie and his colleagues was the first part of a five-year reconnaissance announced by the Minister of Technology in March 1968. It is being carried out by a team of twelve qualified staff on a grant of £250,000. One of the interesting features is the amalgam of techniques

being used to locate the uranium deposits. As much of the area is covered by a layer of peat, detection of radiation by scintillation counters is of only limited value. In addition therefore a technique was used which involved boring into the peat and measuring the concentration of radon in the "ground air". Variations in the radon and gamma ray intensities give an indication of the extent of the uranium deposits, but it is pointed out that probes down to several hundred feet will have to be employed before any firm estimate can be made of the amount of uranium present.

MEDICINE

Watching for Carcinogens

THE International Agency for Research on Cancer has concentrated on environmental cancer biology since it was set up by the World Health Organization in 1965, and its decision now seems to have been an excellent example of foresight. With programmes that cover hazards from asbestos to pesticides, the agency is well placed to feed the massive interest that has since developed in what the environment can (and cannot) inflict on the body, as is clear from its latest annual report. What is more, the report stresses the need to deal chiefly with human disease patterns "as long as our knowledge of comparative carcinogenesis and toxicology in animals and man remains insufficient to permit extrapolation", for "no alternative methods have been established permitting a definite assessment of environmental hazards to man". Those who would cry wolf at the sight of a dead rat might do well to consider this message.

On a budget of just under \$2 million, which is contributed by nine participating states and by WHO, the agency runs its own laboratory in Lyons—where a new building is due to be completed soon—and backs research in national centres; it has set up regional centres in Nairobi, Singapore and Jamaica, and also offers fellowships for training and for travelling. Its aim is to work on international cancer projects which as far as possible do not clash with what its participating states are doing on their own account, although it recognizes its usefulness as a neutral source of second opinions in cases where national sources are controversial or inadequate. In general its role is to gather new information, thus taking part in a convenient division of labour with the WHO Cancer Unit which is responsible for treatment and control.

The epidemiological section of the agency continues to digest statistics and produce uncomfortable correlations. Cancer of the oesophagus in Curaçao seems to be related to the consumption of a hot maize porridge; laryngeal cancer has a high incidence in Thailand and may turn out to be not unconnected with a local cigar which contains uncured home-grown tobacco and tree bark in roughly equal proportions; and there are even hints of a correlation between cancer of the colon and rectum and the consumption of meat. In the future, the environmental emphasis is expected to continue. The agency is hoping to increase the number of participating states, and intends to build up the network of collaborating laboratories engaged in specific programmes with different population groups; but in spite of the training it offers, it is still having trouble in attracting enough good staff to Lyons.

CONSUMERISM

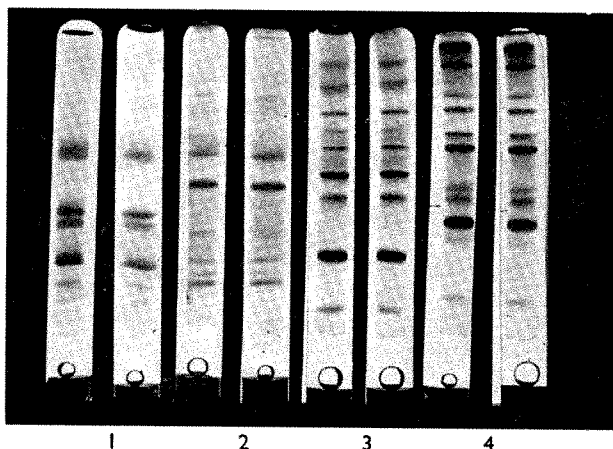
A Fish by Any Other Name . . .

To sell a plaice as a sole may not seem the gravest of crimes, but is nevertheless to be illegal from next year. Techniques for finding out what the species of a fish really is are consequently being made much more subtle, to judge by the report for 1969 of the Torry Research Station in Aberdeen (HMSO, 7s). By an electrophoretic method which identifies the water-soluble proteins from a small piece of flesh, the fish fraud can now be tracked down even if his fish have been skinned, filleted and cooked. For the moment, however, tinned fish will escape this net, because the proteins are changed too much in processing.

Another advance on the human nose is embodied in simplified kits that have been devised at Torry for assessing the freshness of wet fish. Their object is to measure the concentration of hypoxanthine, which begins to form in the flesh of a fish as soon as it is dead; an extract of the flesh is treated with reagents whose colour disappears if the fish is off. Prototype kits are soon to go on trial, but before they can be used regularly the amount of hypoxanthine that is to define an unacceptable fish will have to be investigated more precisely.

Measuring the quality is particularly important for fish that are frozen at sea, because they are sometimes kept frozen for several months. To determine the best way of handling catches, Torry staff have been comparing different methods that are used on board commercial trawlers. Both the delay before the fish are frozen and the temperature at which they are kept seem to be crucial; one promising approach is to use refrigerated sea water to chill the fish first. According to a survey of the consumer end of the fishing industry, however, sea frozen fish are more consistently of high quality than wet fish, and barely more expensive.

About half of the British catch is converted to fish meal for feeding animals. This proportion, however, consists largely of surplus fish and offal from fillets, and the director of the Torry Research Station, Dr G. H. O. Burgess, argues in the report that Britain would do well to develop an industrial fishery, for in 1968 the country imported about six times as much fish meal as it produced. The value of the imports, together with imported fish oil, was £41 million.



Electrophoretic "fingerprints" of fish species: 1, cooked lemon sole; 2, cooked Dover sole; 3, raw lemon sole; 4, raw Dover sole.

Too Much Astronomy at the IAU

IN the end the International Astronomical Union meeting at Brighton was a roaring success. The right level of unobtrusive efficiency soon replaced the casual organization during the registration period that had looked ominous for the 2,300 who attended. Obviously many people at the University of Sussex and the Royal Observatory nearby at Herstmonceux had been taking time off from their research to see that things ran smoothly. Even a readable newsletter was printed daily for the participants.

The danger now is that the union's executive will have been lulled into thinking that general assemblies will always be like this. At the University of Sussex there were often eight meetings, sometimes ten, running at the same time. That sort of thing takes some organizing, and the union may not be as lucky with its hosts another time. As it happened, the question whether the interval between general assemblies should be increased from three to four years was eventually shelved. That would have been no answer anyway to the overlapping meetings, too many papers in too short a time, and overcrowded lecture rooms. Nobody wants to restrict the list of people who attend, but the alternative seems to be to split the general assembly into several symposia. So long as there are astronomers like Fred Hoyle, however, taking part in the sessions on cosmology and on the development of the solar system, subdivision of the general assembly goes very much against the grain. As it is, it is surprising how most astronomers are managing to keep up with all branches of their subject when other sciences have long ago fragmented. At Brighton last week it never seemed odd to see, for example, a radio astronomer attending a meeting on the lunar ephemeris, or an atmosphere scientist listening to the debate on pulsars. The IAU will have to go on with its general assemblies in roughly their present form as long as it can. Now is the time to ask, however, that the number of separate meetings be made fewer to give everyone a fair chance of getting to the meetings they want to hear.

But one thing that was clear from the Brighton meeting was that astronomers hate astropolitics. That was part of the reason why the first meeting of the new commission on cosmology was packed to overflowing—the other commissioners were involved in business meetings. On the question of the interval between general assemblies the buck was passed to the new executive committee. And it was disturbing how the financial implications of having two general assemblies in 1973 instead of one was glossed over. That was the solution to the impasse over whether the IAU should accept a longstanding invitation to visit Sydney, Australia, or a late invitation from Poland to celebrate the Copernicus anniversary with a meeting in Warsaw. By a vote of 28 to 14 the IAU accepted the Australian invitation, but with what is to be called an extraordinary general assembly in Warsaw to appease Poland. The meeting in Poland will follow the main general assembly, in the first half of September 1973, and will be limited to six topics—planetary and galactic dynamics, cosmology, a topic from relativistic astrophysics, the origin of the planetary system, a topic from stellar evolution, and a meet-

ing on the history of astronomy to be held at the birthplace of Copernicus.

It would be impossible to predict what will be the talking points in 1973. No doubt the radio astronomers will still be pressing for better protection for the radio astronomy bands. By then optical astronomy may well have been surpassed in precision by radio astronomy. Pulsars will certainly still be an important topic, because even if the emission mechanism is understood by 1973, it is unlikely that the knottier problems concerning the interior structure of neutron stars will have been unravelled by then. And it will be interesting to see how much more will have been learned about the Moon and planets. It now seems quite on the cards that the Apollo programme will not last long enough to do all that the planetary scientists want.

Astronomy after 1975

IN a pamphlet handed out at the Brighton meeting, the Science Research Council has let slip some of its thinking about the development of astronomy in Britain during the next decade. What is being debated is how to spend the astronomy budget, now standing at around £3 million for ground-based astronomy, when the two big projects now under way are completed. The 5 km aperture synthesis radio telescope at Cambridge will be the first to be finished, in August next year, followed by the 150 inch Anglo-Australian optical telescope in 1974. Still hanging fire, of course, is the project for a 400 foot radio dish to be built in North Wales.

Looking five years ahead, there is a plan to enter the field of astrochemistry with a 30 metre dish able to work from 3 cm down to 1 mm. This is the band containing the molecular lines that have been discovered in the past year or so, and that up to now have been almost entirely an American field. Because of absorption by water vapour in the atmosphere, the dish will have to be on a high dry site—the Americans do some of their observing from Kitt Peak, Arizona. The same constraints hold for the siting of the 60 inch flux collector being built at Imperial College (London), and if the microwave dish ever goes ahead it will be at the same site. At present this looks like being Tenerife.

Now that the scheme for a telescope in Riyadh to be shared by Britain and Saudi Arabia appears to have fallen through, British access to northern skies will hinge on what is being called the Northern Hemisphere Observatory. Funds for this project will not be sought for two or three years. The observatory will have a prime telescope possibly as big as 150 inch with an alt-azimuth mounting, and a second medium-sized telescope. Now that it is accepted that expensive scientific instruments can be built safely outside Britain, site testing in the Mediterranean area has begun. Tenerife is again believed to be in the running.

NEW WORLD

Power, Pollution and Politics

from our New York Correspondent

MAYOR JOHN LINDSAY has now settled for an uneasy compromise, hedged about with all kinds of promises for the future, in the dispute between the City and Consolidated Edison, the utility company supplying metropolitan New York with power. The annual summer power crisis has this year been punctuated by arguments about the application by ConEd to install 1,600 MW of generating capacity at Astoria in Queens, a stretch of industrial wilderness alongside the Triborough Bridge long since abandoned to power stations. Mr Lindsay announced on Saturday last week that he would let the utility company install just half the generating capacity for which it had asked, and enough on the mayor's calculation to assure base-load supplies for the city until 1974. In its agreement with the city, ConEd has also agreed to use natural gas at the new plant if it can obtain supplies, but otherwise low-sulphur petroleum, to push ahead with plans for building nuclear plants, to abandon coal-burning altogether, to import more power by transmission line from outside New York City, to scrub the gases from the new plants and never again to ask for permission for new fossil-fuelled generating capacity. Mr Lindsay may seem to the conservationists to have caved in to ConEd, but if he can hang on he may yet emerge as the man who outlawed fossil fuels from New York.

The long struggle between the environmentalists and ConEd, in which the mayor has sought perhaps unwisely to hold the ring, is not now at an end. There is still dispute about a proposal to build a pumped storage station on the Hudson at a beauty-spot called Storm King Mountain, and although the Federal Power Commission endorsed last week its own earlier decision that the building of that plant could go ahead, there are likely to be several obstacles in ConEd's path before that project can begin. Unlike the Astoria project, Storm King Mountain is intended as a source of power to meet the peak demand for electricity. It was first suggested in the early sixties, but even then evoked protests that the Hudson had been sufficiently despoiled already.

In the battle over the Astoria plant, the environmentalists have been led by Mr Jerome Kretchmer, the Environmental Protection Commissioner for New York. ConEd estimates that by 1974 the peak power need for New York will be more than nine million kilowatts, increasing to nearly ten million by 1975. With 1,600 MW from Astoria it would be able to generate 12.5 million kilowatts by 1974 and another 800,000 by 1975. This would give the city 36 per cent reserve above the projected peak in 1974. ConEd claims that this large reserve is necessary because of the high likelihood of failure in one or more plants. Already this summer two generating plants have failed, reducing capacity by 17 per cent.

Mr Kretchmer and others refused to accept ConEd's arguments, noting that the Federal Power Commission considers reserves of 20 to 25 per cent to be ample. Mr Kretchmer argues that other power

sites are available, but that ConEd does not want to spend the extra money needed to bring the power into the city. He also believes that ConEd could continue to meet any shortages by bringing in power from other utilities, but ConEd responds that other utilities are also facing possible shortages and cannot guarantee any supply to New York beyond 1972.

The chief support for the opposition, however, centres on the dangerous level of air pollution in the city. Astoria is recognized as the most polluted area in the country, and New York itself in recent years has far outdistanced Los Angeles in terms of smog and air pollution. While cars are now considered the major source of air pollution, power plants are not far behind. ConEd claims that the modern plant at Astoria would meet all Federal, state and city health and environment standards; because it also would be replacing older facilities it would in effect decrease the total amount of air pollution in the city. Unfortunately, this will not help the residents of Astoria.

Mr Lindsay's plea that ConEd should use natural gas at Astoria if only supplies can be found is an attempt to reduce the emission of sulphur dioxide and smoke, but there is now such a grave shortage of natural gas that ConEd recently joined with a number of conservation groups in petitioning the Federal Government for a larger supply of natural gas to meet the present needs. In any case, both gas and oil present health hazards. Mr Kretchmer estimates that between 1,000 and 2,000 people die prematurely in New York City each year from sulphur dioxide poisoning; and even if Astoria used low-sulphur oil the plant would add about 15,000 tons of sulphur dioxide to the air annually according to Mr Kretchmer.

Natural gas also has its snags, chiefly that it produces nitrogen oxide, a cause of smog. At a symposium last week sponsored by the Citizens for Clean Air Dr Carl Shy, an epidemiologist at the National Air Pollution Control Administration, estimated that the nitrogen oxide from the Astoria plant would cost society between \$250,000 and \$750,000 a year, the cost of increased respiratory illness among the local residents.

Whatever the truth, the debate has amply demonstrated that power has never been properly planned in the city. The Astoria plant appears to be an inadequate, and probably dangerous, solution to the present crisis, but the philosophy behind it is even more questionable. Until recently it was assumed that the city, like the rest of the country, could continue to expand simply by increasing all inputs. But services in the city have begun to disintegrate in recent years, possibly through poor administration, but certainly through overloading and strain. The problem facing Mayor Lindsay and ConEd should not be how to provide more power, but how to use what is available more efficiently. The way things are going, the need for more power will certainly disappear as more people desert the city for the still somewhat clean air of the suburbs.

AEROSPACE INDUSTRIES

Lockheed Lives Again

THE project for building the huge military transport aircraft called the C5A has survived yet another attack by critics in the Senate of this and other new weapons systems, with the result that the Lockheed Company will not find itself having to abandon manufacture of the aircraft for lack of funds. It remains, however, to be decided how the relationship between the Department of Defense and the luckless company will eventually be resolved. Already there are reports that a part of the price that Lockheed will have to pay for being bailed out of its contract for development and manufacture of the aircraft will be the transfer of the Georgia plant at which the work is concentrated to public ownership.

The C5A has become in the past few months a haunting admonition for all industrial concerns anxious to win military contracts. In technical terms, the aircraft is a remarkable development in its own right—it is designed to lift more than 80 tons of military equipment. Much of the engineering interest of the development has been the design of a wing sufficiently large and efficient to provide the necessary lift. Administratively, the contract for the development work, originally signed in October 1965, broke new ground by being one of the first fixed-price contracts of this size negotiated by the Department of Defense as part of Mr McNamara's belief that it should be possible to save public money by the introduction of commercial procedures into military procurement. The original contract supposed that \$1,900 million would cover the cost of development and the manufacture of 115 aircraft. At the beginning, Lockheed must have been given some assurance that the project would not become a very heavy loss by the provision in the contract allowing for a price increase for the second half of the production run if the estimated costs should have turned out to be too low in the production of the first 57 aircraft. In the event, the complications of the wing design and of the electronic equipment built into the aircraft have plainly turned out to be much greater than originally supposed, while the Pentagon seems to have set its face against a loosening of the tight performance specification originally agreed on. On the present showing, it seems as if the cost of the whole enterprise would turn out to be close on \$5,000 million, although the total cost was for practical purposes reduced last October when the Air Force said that it would settle for 81 aircraft rather than the 115 originally ordered.

This reduction of the scale of the operation seems to have aggravated Lockheed's troubles. Under the original contract, it would have been possible to offset losses on the first half of the production run by increasing the price charged to the Air Force for the second half of the order, although there was an upper limit to the extent to which Lockheed could expect to make good its losses in this way. With the curtailment of the second series of aircraft ordered, the scope for recovery in this way has also been curtailed, and it seems to be agreed that Lockheed will make a loss of somewhere between \$200 million and \$650 million on the whole operation, depending on the outcome of current negotiations with the Department of Defense.

The Lockheed issue came up in Congress last week in the form of an amendment to the Military Appropria-

tions Bill put forward by Senators Proxmire and Schweiker, two redoubtable critics of military expenditure. Their object, on August 20 and then on August 26, was to limit the freedom of the Department of Defense to spend a sum of \$200 million set aside in the C5A appropriation for what are called contingencies. This sum of money is additional to the \$334 million estimated by the Air Force to be due to Lockheed under the terms of the contract in the current financial year, and which is thought sufficient to complete some 42 aircraft. The assumption is that the contingency allowance will in practice be used to make good some of the company's expected losses. The arguments for making these payments, put forward last week in the Senate, turn around the strategic advantages of the C5A and the likelihood that too fierce a view of the situation might force Lockheed to default on its contract or even to file for bankruptcy. In the event, the Senate agreed that the \$200 million should be spent, which gives the Pentagon an opportunity to find a way of keeping production going. From last week's debate, however, it is clear that there are influential voices in the Senate against too lenient a settlement. Whatever happens, there is a feeling that defence contracting will never be the same again.

DEFENCE POLICY

ABM Undeterred

As everybody expected, there was no fight in the Senate when eventually Senator Brookes's attempt to confine the deployment of the Safeguard to the two sites at which deployment has already begun came up for debate. Unlike the Cooper-Hart Amendment to the Military Appropriations Bill (see *Nature*, 227, 770; 1970), the Brookes Amendment would have allowed the Department of Defense to spend all the Safeguard money requested for 1971, but would have compelled the diversion of the \$300 million odd allocated to the preliminary work at the Whiteman base in Missouri to the improvement of the radar defences at the sites in Idaho and North Dakota. Inevitably, the argument turned not so much on the political question of whether the work being carried forward on Safeguard will help or hinder the SALT talks, due to be resumed in Helsinki in November, but on the admitted inadequacy of the radars being put in at the Safeguard bases. During the financial year that has now begun, the Department of Defense intends to equip the two existing sites with extra Sprint missiles, so as to help ensure the defence of individual Minuteman silos, but Dr John Foster, Chief of Research at the Pentagon, agreed earlier this year that a sound defence of these missiles would require point-by-point radars as well. It is entirely understandable that the Senate should seem to be on weaker ground when trying to force new patterns of technical development on the military than when arguing about general principles, but last week's debate nevertheless provided a fresh insight into the ways in which the Safeguard system is being put together from its separate components.

Eventually, there will be twelve missile sites, covering the United States uniformly. At each of the sites will be a local radar system with a range of several hundreds of miles, and at seven of them will be Presimeter Acquisition Radars with ranges of several

thousands of miles. Both radar systems consist of phased arrays of antennae mounted on the exterior walls of concrete bunkers several hundreds of feet in their dimensions above ground. For practical purposes, the long range radars will control the flight of the long-range Spartan missiles, the first of which are to be tested in action (against dummy ICBMs) this summer. The shorter-range radars will control the Sprint missiles, designed for the rapid acceleration necessary if comparatively local interception is to be successful. What seems now to have been established by those critics of the system who say that Safeguard will not function well is that the shorter range radars may be saturated by a sufficiently coordinated enemy attack or by the use of multiple warheads or even dummy warheads. What Senator Brookes was arguing last week was the need to supply this extra equipment so as to make sure that some at least of the Minuteman sites are properly defended. His case was defeated fifty-three to forty-five.

AGRICULTURAL GENETICS

Corn Blight hits Male Sterility

THE corn blight which has already damaged crops in the southern states in the past few weeks has now reached Iowa and other states in the mid-West corn belt, throwing the commodities market into confusion and causing consternation as far away as Washington. Until the growing season is over, some weeks from now, it will be hard to tell whether the yield of corn—maize to many people outside the United States—will be reduced by the five per cent or so which the more cheerful agronomists are talking about, or whether a quarter of the crop will then be found to have disappeared. In Chicago, the commodity markets have already responded by increasing the price of corn by something like 20 per cent. An Australian entomologist, back last week from Iowa, takes the more gloomy view, saying that "they should have seen this coming all along".

The fungus responsible for the blight, *Helminthosporium maydis*, has been known in the southern states for the best part of a century. In the past twenty years, however, its effects have been kept in check by the development of resistant varieties of corn. What may now have gone wrong is that the male-sterile varieties of corn now widely planted in the United States have, by their genetic stability, provided an especially vulnerable target for a recent mutation of the fungus. If reports that the male-sterile varieties are now suffering most severely from the blight should be confirmed in the next few days, the plant breeders who have recently been boasting of the virtues of male-sterile varieties may find themselves pilloried, not praised.

Corn plants are bisexual, with the male reproductive organs carried on tassel-like appendages. In what have become the old days, farmers would attempt to ensure cross-pollination of corn plants by removing the male appendages from alternate rows of corn plants—a costly procedure. With the emergence in the past few years of varieties with improved yield and disease resistance, there was also a natural welcome from the emergence of a strain of corn from the plants of which the male reproductive organs would be discarded before maturity, and this is now the most commonly

planted variety of corn in the United States. For a time, at least, the lack of cross-pollination and the genetic stability which was the natural result seemed to be a great advantage. The same genetic uniformity may now, however, account for the vulnerability of the corn crop in the Middle West and the South. Certainly the fungus, first noticed in its new form only a year ago in Illinois, has spread like wildfire. For the plant breeders, the problem is what to plant next year. Corn farmers may yet find themselves detasselling their plants.

DEMOGRAPHY

Population Explosion Falls Flat

THE falling trend of the birth rate during the sixties has now prompted the Census Bureau of the Department of Commerce to publish new estimates of the population of the United States in the decades ahead. On present trends, the population in the year 2000 is estimated to be 281 million, or a little less than the lower bound for the year 2000 put out as recently as 1967 by the Census Bureau. These substantial fluctuations of the official estimates are not so much a sign of poor arithmetic at the Census Bureau but of the inherent difficulty of estimating the fertility rate of women, both as the gross figure for the average number of children per woman in the population and as a function of the age of the child-bearing population.

As in the past, the Census Bureau has carried out forward projections for each of several assumptions about the fertility rate for women ranging from 3.35 children per woman (for women not yet of child-bearing age) to 2.45 children per woman. The fertility of American women in the sixties appears to be close to the lower of these limits. The new projects also include a forward projection based on the assumption that the completed fertility rate of American women works out at 2.11 children per woman, the ratio necessary to maintain the population at a constant level (which would amount to a population of 276 million in the year 2035 and thereafter). Each of the assumptions about gross fertility rate is accompanied by a different set of assumptions about the timing of births and for practical purposes it is assumed that decreased fertility is accompanied by later child-bearing. The unexpected character of the present low fertility in the United States is confirmed by the way in which it has been necessary for the Census Bureau to go back to the Great Depression for information about the timing of births in periods of low fertility, a somewhat hazardous procedure. It has also been necessary to make assumptions about the rate of immigration, and the Census Bureau has settled either for a conservative 400,000 souls a year or, in its attempt to estimate the pattern of a stable population, for none at all.

With these assumptions, the upper and lower bounds of the population twenty years from now are put at 277 million and 255 million respectively, compared with the 203 million now estimated for the population of the United States. If the fertility of the population should however fall merely to the replacement level and if immigration is brought to a halt, the population of the United States in 1990 will be 248 million—a comparatively small reduction from the lower bound corresponding to the present level of fertility. The

forward projections now published consist not merely of total population as a function of time but of the age distributions corresponding to each set of assumptions, and it is no surprise that the least quickly growing or even static populations are characterized by smaller proportions of younger people.

Although the new calculations are no doubt a valuable correction to the estimates published three years ago, outsiders may well ask whether enough is yet known about the causes of the decline in fertility in the sixties for any forward projections to be meaningful at this stage. In the sixties, the crude birth rate (births per year per 1000 population) fell from 23.9 in 1960 to 17.6 in 1968, and there is as yet no sign that it will be halted. Even the most pessimistic of the forward projections, however, implies that there will be an increase of the crude birth rate in the seventies and early eighties. On present form, there is very little sign of such a tendency. At the same time, however, it is only natural to ask whether the recent rapid decline of the birth rate represents a reduction of the fertility of women or, alternatively, a drastic change of child-bearing pattern such as might be constituted, for example, by a postponement of child-bearing to a later age. Even the results of the 1970 census, now being analysed, are unlikely to throw much light on these and other possibilities; from which it follows that the embattled Census Bureau is likely to find itself putting out yet another correction of its forward estimates before many years have gone.

METEOROLOGY

Explosions make it Rain

EARLIER this year the United States celebrated the hundredth anniversary of the American Weather Service, set up largely as a result of prodding by Professor Increase A. Lapham of Milwaukee after storms on the Great Lakes in 1868 and 1869, during which more than five hundred seafarers lost their lives. Now, in 1970, the American Weather Service must be the best equipped in the world. For an outlay of about five hundred dollars anyone can build the equipment to pick up photographs from the automatic transmission cameras on American meteorological satellites. Forty-five national weather services are known to be taking these pictures. Satellites are being launched into geostationary orbits so that, fixed at one point above the equator, they can follow storms as they develop. Cloud cover can now be measured over the night-time half of the Earth using infrared sensors. As much as anything, the satellites mean that data can now be recorded over regions that are otherwise virtually inaccessible except at great expense—most of the oceanic areas of the southern hemisphere, for example. Because of the satellites the US Navy no longer has to station weather ships along the air routes from New Zealand to the bases in Antarctica.

A celebratory history of the Weather Bureau has now been published (*A Century of Weather Service*, by Patrick Hughes; Gordon and Breach, 1970). As much as anything the book is fascinating for its illustrations, photographs of the pioneers of meteorology in the United States and their equipment. But the history of meteorology has not been entirely a success. Efforts to modify the climate artificially have had equivocal success. The emphasis has been on

inducing rain, whereas in the Soviet Union, the other major centre of this kind of experiment, comparable effort seems to have been spent on making the weather as pleasant as possible. In the United States there have been a variety of experiments since 1945, but as long ago as 1890 Congress appropriated money to General R. G. Dyrenforth to find out whether explosions cause rain. A series of unsuccessful tests in 1891 and 1892 using batteries of cannons, and bombs with time-fuses, carried by balloons and kites, culminated in the firing of balloon bombs at forty-five minute intervals from noon until 3 a.m. the following day, after which it indeed began to rain on the sleepless inhabitants of San Antonio.

SPACE BUSINESS

Disputes about Satellites

THE National Aeronautics and Space Administration seems to be going through a bad patch in its attempts to award contracts for the development of specific Earth satellites. Earlier this year, the award of a contract to the General Electric Company for the development of a Technological Satellite was challenged and eventually cancelled (see *Nature*, **226**, 402; 1970) on the grounds that the company had an unfair advantage over its competitor, the Fairchild-Hiller Corporation. Now it appears that a similar complaint against the award of a contract to General Electric for the development of an Earth Resources Satellite has again been made by the unsuccessful bidder, TRW Inc.

The dispute on this occasion seems to centre on the degree to which the two companies were given access to a preliminary study of the design of the system carried out by the Bendix Corporation. It seems to be agreed that both the bidders in the competition were sent copies of this report only a few days before their proposals were due at NASA, but that the General Electric Company worked with the Bendix Corporation on the preparation of its own proposals. On this occasion, it appears that NASA is satisfied that there is no need of a formal reappraisal of the award of the contract.

Foreign Visitors

THERE is some consternation at Stanford University about the decision of the Czechoslovak Academy of Science that Dr Jiri Masek, head of the department of inorganic chemistry at the Polarography Institute at Prague, should not be allowed to take up a National Science Fellowship at Stanford. According to a statement by the university last week, Dr Masek has been informed that the academy considers that the numbers of Czech scientists working abroad now exceeds "a reasonable limit", and that until some of those working abroad return home, others will not be allowed to leave. Dr Masek has been told, according to Stanford University, that he should keep on applying for permission to work at Stanford. Professor Henry Taube at Stanford, with whom Dr Masek would have worked, says that he is prepared to wait for him.

Some Pathologies of the Scientific Life

(Extracts from the Presidential Address by J. M. Ziman to Section X of the British Association Meeting)

A COLLECTION of pathological cases must inevitably seem like an attack on the whole body of science. In the present irrational mood of our intelligentsia, it would seem folly to add more fuel to the fires on which they are mentally roasting us—with our books piled high about us and our instruments stuffed down our gullets. Let me say, then, most emphatically, that I do *not* believe that the internal state of the scientific community is desperately unhealthy.

One of the characteristics of modern science that many people find scandalous is the political in-fighting that breaks out when big administrative decisions are to be taken, and big money is to be handed out. We rightly expect distinguished scientists to be honest, disinterested, courteous, etc., in the laboratory or lecture hall—just as we expect doctors to be humane and painstaking in the consulting room or operating theatre. But the general administration of a large scale organization, whether a scientific research establishment or a hospital, is not in itself a scientific or medical activity, and demands an entirely different code of behaviour. It would be pathological if this aspect of the scientific community were *not* subject to the general sociological laws of bureaucratic and political systems.

I want to discuss those occasions when the *internal* social and psychological mechanisms of scientific research break down. Science is a communal activity, directed towards the creation of a rational intellectual consensus. Scientists are in tacit cooperation when they communicate their results to one another, criticize, recognize, refer to, appoint or promote each other. They act in the expectation that their contemporaries will behave according to certain conventions. Any serious breach of these conventions is a pathological symptom, deserving our attention.

The typical psychopathology of ordinary society is crime. Strangely enough, deliberate, conscious, fraud is extremely rare in the world of academic science. One would have thought that the material rewards of research were tempting enough—cosy university appointments, research grants galore, plenty of conspicuous travel—to encourage a noticeable amount of scholarly dishonesty. Yet the only well-known case is "Piltdown Man", which is more of a monument to the absolute trust that we have in a reputable fellow scientist than an example of a grandly conceived crime. I suppose there must be a fair bit of lazy cheating—experimental data filled in by Guess and by God, or theories stolen from little known works and plagiarized—but I have never come across it myself.

One must not get the idea that scientists are all that admirable in everyday life. As I have already remarked, they will intrigue for political ends like any Jesuit, and can be as lordly as any consultant physician in the control of their juniors. They can deceive their wives, fiddle their tax returns, drive drunkenly, live beyond their means, feed parking meters, beat their children, and otherwise behave as antisocially as anyone else when the occasion demands. There are scoundrels amongst scientists, just as there

are amongst lawyers, or architects, or naval officers. But I have a very strong impression that these traits are repressed within research itself.

Perhaps the rewards are too meagre, and the risks of being found out are too great. It may be, also, that the long training of the professional scientist conditions him against conscious deceit. Those years leading up to the PhD, the struggle to obtain useful results, to master the theory, to write it all down, and to please his supervisor, are a tough discipline. If he has studied in a good institution, then he will have internalized very high standards of honesty, scepticism, and criticism, so that he will never find it easy to let his mind slide over the difficulties and objections, and thus to rationalize a really crude deception. He should have learnt also how completely each scientist relies upon the sincerity and good faith of those whose results he quotes as evidence for his own conclusions. Unwitting error causes trouble enough: the suspicion that any paper we refer to might be fraudulent would completely paralyse further research. A fierce and uncompromising honesty is one of the standard attributes of the so-called "scientific attitude". I do not think it is inborn, but it is certainly moulded into one by powerful social pressures in the graduate student phase of one's career.

Self-deception, on the other hand, is an exceedingly common phenomenon in the scientific world. We are all, of course, familiar with extreme cults, such as Flying Saucers and Extra-Sensory Perception, on the fringes of genuine science; it is important to realize that the reputable scientific literature abounds with similar, if less obviously fantastic, irrationalities.

What does this type of pathology signify for the scientific life and the scientific community? It is not surprising, perhaps, that the initial claim to some extraordinary discovery should get published. It is the duty of every journal editor and referee to have an open mind towards completely new effects, and to suspend his scepticism temporarily in order to entertain the possibility that some highly speculative and incomprehensible theory may, after all, be correct. But how are we to explain the very large number of papers that often then appear, both in support and refutation of some such preposterous proposal? The truth is, perhaps, that scientific progress is difficult and uncertain. The hope of participating in an important scientific revolution encourages many able professionals to follow up the initial claim. A considerable amount of experiment to establish negative results is then required to debunk the effect or theory. The journals must be kept open to such communications for a year or two, and cannot easily refuse to publish the work of the master and his remaining disciples, who can give respectability to their papers by referring back to the earlier published literature.

The conflict of ideas, results, claims and theories is basic to scientific progress, but there are powerful norms forbidding intense dispute, personal abuse, alienation and fragmentation into mutually irreconcilable "schools". If knowledge is to be "consensible" then division into groups that do not share common

aims, and do not speak or listen to one another, is the negation of science, and must be avoided at all costs. Controversy of this violence, which does occasionally occur, must be regarded as deeply pathological.

The psychological balance of scientific innovators is exceedingly delicate. One must have sufficient confidence in one's own notions to carry conviction in argument. Yet one must not become so deeply committed that one cannot escape from them if they prove untenable. *The Double Helix* brings out the passion and anguish with which scientific research is really pursued. Kubie remarks that "the structure of science adds layer on layer, each burdened by more subtle and complex unconscious emotional investments, demanding of the scientist an ever greater clarity about the role of his own unconscious processes in his conscious theories and experiments, and each requiring an ever more rigorous correction for the influence of unconscious preconceptions". I would add that the "norm" demanding impersonality of expression—for example, the use of a neutral tone and passive voice in scientific writing—is more than a rule for protecting the scientific community from violent disputes; it is a device for warning us of the personal psychological danger of allowing our private passions to take control of us in public.

Intellectual property is, after all, so intangible and evanescent. An original theory or discovery that is acclaimed today may turn out tomorrow to be an error or a blind alley. The question of ownership is much more uncertain than with patents or copyrights; absolute priority of discovery is meaningless within a slowly evolving complex of ideas; yet a single sentence might be enough to convey the essence of a great revolution of thought. Even the most successful scholar is aware of the danger of losing all the intellectual capital that he has so laboriously amassed. An attack upon his point of view threatens his whole reputation and self-esteem: he sees his name in the history books being erased from the column of heroes, and put in a footnote amongst those tiresome fools who have wasted the time of their contemporaries with their errors and irrelevancies.

The pathological phenomena to which I would draw attention are the contrived, and yet almost fortuitous, titles of eminence within the very top ranks of the scientific community. A few hundred Nobel Laureates are not enough to go round amongst the several thousand living scientists who are, one would say, in that class of ability. The same applies to membership of the National Academies—even to our own dear old Royal Society—which have not expanded in numbers to match the growth of the scientific community. Election becomes more difficult—and the disappointment of reasonable expectations is that much more bitter. I fully appreciate the difficulty of any public discussion of this sort of thing, but I think we must admit that it is unhealthy when we can point to a number of first class scientists, still in the prime of their powers, whom every one agrees to be fully of the supposed standard, and who are yet unlikely now to be elected. The function of the Royal Society, and of similar Academies, is to be a meeting place and organ of opinion for *all* the most responsible and senior scientists in the country, and not just a prestigious labelling machine for those who have been fortunate enough to get their noses slightly ahead at the right moment in the race.

Am I insisting, puritanically, that the scientist should lead a lean and austere life? What I mean is not that we should all live like hermits, but that great affluence should not come too easily. It is difficult to point to specific and scandalous failures—yet one must be aware of a certain flabbiness in the intellectual world due to the enormous subsidies it has had to digest. In recent years, it has become just a bit too easy to get a PhD of sorts, and an academic job of sorts, and a grant for research of sorts—to produce yet more PhDs of the same sort. Scientific research is not simply a professional job, to be entered into in the spirit of plumbing or accountancy. Nor is it a mechanical activity, like programming a computer, that can be learnt from a manual. It demands a sense of vocation and dedication; it is an art form, transmitted from master to apprentice by oral tradition and imitation over a period of years. To expand the scientific labour force more quickly than it can be adequately trained must surely lower the quality of the product. The current shake-out in the PhD job market is not, to my mind a symptom of some serious disease, but rather an occasion for a nature cure by gentledieting.

The fact is that the academic community, despite its tendency to look inward and not care much about what the world is doing, is quite easily bought and sold. Dons may not be very ready to undertake the dirty work of Industry and Defence, but they are quite willing to be subsidized to any extent, in the name of basic research, etc., by those whom they despise.

My other example of a pathological—indeed totally destructive—effect of social pressure on organized science comes from the political pole—from the Soviet Union. I refer, of course, to the tragic phenomenon of Lysenkoism. We must all be grateful to Dr Zhores Medvedev for writing, and allowing to be translated and published abroad, his vivid and authentic history of this terrifying aberration in a country supposedly dedicated to rational scientific materialism. The terrifying feature was the apparent normality of the scientific organization within which this false doctrine existed. The tragedy lies not with those few who were forced, by irresistible threats of violence, to keep silent or to say the opposite of their thoughts: it is with the many who seem to have accepted the doctrine as it was taught to them and failed to subject it to their own tests of rationality. We cannot blame a half-educated crank for believing his own theories and trying to get them accepted: we must ask what was wrong with a whole scientific community that it allowed itself to be captured by such crazy notions.

It is easy enough for us to say that it could not happen here. We should, indeed, be singularly unfortunate to have full-blooded Stalinism wished upon us. I hope that at least a few of our own scholars would then behave as heroically as some of the Russian geneticists in those terrible years. But I think we can learn, from this as from the other pathologies I have discussed, some positive facts about the scientific community and the way it really works. In our own democratic and liberal social environment, we take for granted such elementary features as freedom of speech, freedom to travel, permanent tenure of academic office, an independent diversity of universities and other institutions, which are the real guarantees of healthy, productive science.

NEWS AND VIEWS

Après Temin, le Déluge

It is a measure of the excitement invoked amongst molecular biologists by Temin's discovery that RNA tumour viruses can act as templates for the synthesis of DNA (see *Nature*, **226**, 1198; 1970) that no less than ten pages of this issue of *Nature* are filled with reports about the mode of action of these viruses. In the brief time that has elapsed since Temin announced in June that RNA tumour viruses contain an enzyme activity which requires RNA and all four nucleoside triphosphates to synthesize DNA, a flood of research workers have cast covetous eyes on the pickings to be gleaned from Teminism (see *Nature*, **227**, 887; 1970).

The immediate product of the RNA-dependent DNA polymerase enzyme activity found by Temin seems to be a double-stranded hybrid nucleic acid, the result of synthesis of a complementary strand of DNA on part of the viral RNA template. In an impressive burst of activity, Spiegelman and his colleagues at Columbia University have characterized this hybrid, and have shown by hybridization studies that the DNA strand is indeed complementary to the viral RNA (*Nature*, **227**, 563; 1970). On page 1026 of this issue of *Nature*, Green and his colleagues at St Louis University observe that the buoyant density of the RNA-DNA hybrid synthesized *in vitro* is close to that of the viral RNA itself; this implies that only a rather small part of the RNA genome is taking part in the synthesis of complementary DNA. It seems likely that rather more of the viral RNA directs DNA synthesis *in vitro*.

What is the purpose of this unusual information reversal? Evidence is now mounting to support the view that formation of the RNA-DNA hybrid is just a first step in the synthesis of a double-stranded DNA carrying the genetic information of at least some of the genes of the RNA tumour virus. Such a DNA would be able to integrate into the genome of its host cell (it is tempting to draw an analogy with the integration of bacteriophages into bacterial chromosomes); this integration would explain how RNA tumour viruses can stably transform their hosts. It seems unlikely that the RNA-DNA hybrid itself could fulfil this function, and on page 1029 Spiegelman *et al.* report the discovery of a second DNA polymerase activity in the virion which is probably concerned with the production of duplex DNA.

This enzyme, at least, is conventional in its mode of action and uses DNA as template for the synthesis of further DNA. It replicates double-stranded DNA well but works less efficiently with single-stranded DNA templates. Because all the DNA-dependent DNA polymerases found previously prefer single-stranded templates, this enzyme is interesting in its own right,

as well as for the part which it must play in viral infection. This may be the reproduction of the duplex DNA carrying viral information to increase the chances of a successful integration into the host cell chromosome.

But where does the double-stranded DNA template come from for this enzyme to act on? Presumably, the virion must contain yet another enzyme activity to convert the RNA-DNA hybrid to a duplex DNA. This might be the synthesis of a new DNA strand to displace the RNA strand. It seems likely that this activity is not vested in a third enzyme, but is another function of the DNA-dependent DNA polymerase, for Spiegelman has found (see *Nature*, **227**, 887; 1970) that poly dC · poly rG is a remarkably good template for this enzyme.

Demolition of the hallowed idea of the molecular biologist that RNA cannot synthesize DNA has prompted a reinvestigation of other ideas. It has been claimed for some time that RNA tumour viruses do not contain any DNA, but Levinson *et al.* (page 1023) and Green and his colleagues (page 1026) have taken another look at this question, and find that there is a small amount of DNA in the virion. Levinson *et al.* find that it comprises two components, one which cosediments with the RNA viral genome at 65S, the other being a small unit sedimenting at about 4S. The DNA made *in vitro* by their preparation of viral DNA polymerase sediments very similarly, but it is not yet clear whether this is a coincidence or whether this DNA has been synthesized by the viral enzyme for some purpose.

The existence of biochemical mechanisms which synthesize duplex DNA from the viral RNA suggests that this process must play some part in the action of these tumour viruses; but what direct evidence is there that viral gene functions are really needed for transformation? On page 1021, Martin reports the isolation of a temperature sensitive mutation in a function of the Rous sarcoma virus which is needed for transformation of the host cell. The mutant strain is normal at 36° C, but although it grows normally when the temperature is increased to 41° C, the infected cells retain their characteristic morphology; they are not transformed. So the mutant function must be concerned with host cell transformation, but is not needed for the growth of the virus. Even more significant, when cells which had been transformed at the lower temperature were increased to 41° C, they gradually reverted to the appearance characteristic of normal cells. This implies that the continued expression of viral genes is needed if the host cell is to be maintained in a transformed state. And this is most simply explained if those genes are integrated into the genome of the host.

PROTEIN SYNTHESIS

Translation not Universal

from a Correspondent

NEW information is still being obtained about various aspects of the translation process, during which genetic information carried on messenger RNAs is used in the biosynthesis of specific protein molecules. It is becoming increasingly clear that in spite of the universal nature of the genetic code, different parts of the translation apparatus are not entirely interchangeable between different organisms. In the past, various workers have synthesized the globin portion of rabbit haemoglobin *in vitro*, using with gay abandon unwashed ribosomes from rabbit reticulocytes (carrying the messenger RNAs for the globin chains and the factors necessary for protein synthesis) together with aminoacyl tRNAs from *Escherichia coli* or yeast. Because the primary structures of the globin chains are known and the system has several technical advantages, it has proved particularly useful for exploring the degeneracy of the genetic code.

But, in a careful study, Hunter and Jackson (*Europ. J. Biochem.*, **15**, 381; 1970) have re-examined the products actually obtained when globin is synthesized in this way, and sadly conclude that substantial levels of miscoding occur during translation in this heterologous system. They demonstrated this by using tRNA charged with radioactive amino-acids, astutely choosing three amino-acids (cysteine, methionine and valine) which occur rather rarely in the globin molecule, so as to be able to study where these were incorporated within the protein. They mixed the radioactive globin which was synthesized with carrier amounts of natural rabbit globin, and fingerprinted tryptic digests of the mixtures. The fingerprints were stained with ninhydrin to reveal the peptides arising from the carrier material and then autographed to find out which peptides contained the radioactive amino-acids.

In all cases, they found that substantial amounts of the labelled amino-acids occurred in peptides not normally expected to contain them. In some instances peptides with altered mobilities were produced by the amino-acid changes. In a few surprising cases they found that peptides which ought to have contained labelled amino-acids did not, and they were unable to explain this entirely satisfactorily although they wondered if some residual reticulocyte tRNAs from the unwashed ribosomes were competing very effectively for these sites. A judicious control experiment demonstrated that, under identical conditions, incorporation from reticulocyte valine tRNA was found only in the true globin peptides. From their results they were able to deduce that the cysteine tRNA (which normally recognizes UG_C^U) was also probably reading the arginine codons CG_C^U because of mismatching of the codon-anticodon complex. Hence in five cases cysteine was introduced in place of arginine. They also suggested that both the methionine tRNA_F and tRNA_M were extensively reading GUG, and that methionine was being introduced instead of valine into various points in the molecule. Hunter and Jackson believe that the most probable explanation of these somewhat traumatic results is that the reticulocyte ribosomes are unable to position all the *E. coli* tRNA species correctly relative to the codons on the

mRNAs, presumably because of nucleotide sequence differences between the tRNAs from *E. coli* and reticulocytes.

Some important results from the wonderful world of factors are reported by Revel and his colleagues in two recent papers (*FÉBS Lett.*, **9**, 213 and 218; 1970). The attachment of mRNAs to ribosomes in *E. coli* is known to depend on the presence of three protein factors A(F1), B(F3) and C(F2). Factor B is necessary for the recognition of natural mRNA templates, as opposed to synthetic polynucleotides. They find that factor B is heterogeneous, and can be split into multiple components by chromatography on columns of DEAE-cellulose and hydroxylapatite. These fractions are distinguished from each other by preferentially stimulating the translation of different mRNAs *in vitro*. Thus one of the fractions stimulates the translation of bacteriophage MS2 RNA, while the other activates the translation of bacteriophage T4 mRNA (assayed by the product of translation of the lysozyme cistron). The two activities were not entirely separated from each other, and it is unclear whether this is because of incomplete purification, or whether each really possesses some activity in the other system. The fraction stimulating the translation of MS2 RNA could be further split into at least three peaks. They elegantly demonstrated that one of these peaks caused preferential translation of the coat protein cistron. Further evidence as to the regulatory role of the B factor fractions was obtained by studying cells infected with bacteriophage T4. After infection, the stimulatory activity of factor B for the translation of T4 mRNA, and particularly the late messenger, was considerably increased, while factor B activity in MS2 translation was diminished. These findings suggest that a further mechanism governing the control of gene expression is operating at the translational level. It will be important news indeed if analogous systems are characterized in eukaryotic cells.

PROTEIN SYNTHESIS

Jumping on the Bandwagon

from a Special Correspondent

If the meeting on mammalian protein synthesis held at Cold Spring Harbor last week had been held six months ago, almost all of the thirty-three contributions would have been dramatic news. But research has progressed so fast that by now, however, the topics discussed seem hackneyed and weary; as repetitive report succeeded report, it seemed at times that the predominant view of participants was that the only problem worth solving in molecular biology is how haemoglobin synthesis is initiated in reticulocytes.

Haemoglobin turns out to be initiated in a way very similar to bacterial proteins; as D. Wilson (Cornell University), R. J. Jackson and A. R. Hunter (University of Cambridge), A. Yoshida (University of Washington) and H. F. Lodish *et al.* (MIT) showed, a special initiator tRNA (tRNA_i^{met}) provides a methionine residue to start the globin chain. This methionine is cleaved from the protein when the growing chain is about twenty amino-acids long, perhaps because it is then no longer protected by the ribosomes and

becomes available to an aminopeptidase (although this enzyme has not yet been identified). The only difference from bacterial protein synthesis is that the methionine does not have its free amino group blocked by a formyl group.

Mammalian systems for protein synthesis *in vitro* still remain rather unsatisfactory when compared with the more sophisticated systems that can be obtained from bacteria. One difficulty with reticulocytes, for example, is that endogenous mRNA cannot be washed off the ribosomes. It is perhaps a tribute to faith that the mechanisms of protein synthesis are universal that so many reports (and in particular those from MIT) announced the establishment of heterologous systems, in which the various parts of the protein synthetic apparatus are derived from different organisms. This usually means that *E. coli* ribosomes and/or tRNAs are used to translate an alien messenger. Haemoglobin mRNA and the mRNAs of animal viruses are popular templates to use in such systems, although they are rarely translated with the fidelity that prevails *in vivo* (see page 999 of this issue of *Nature*). For example, D. M. Reksoh *et al.* (MIT) have found that poliovirus RNA can be translated into virus specific products by *E. coli* extracts, but initiation takes place at between eight and fourteen sites.

This contrasts with the bizarre translation of poliovirus RNA *in vivo* into one large protein which is only subsequently cleaved into individual protein entities. But more encouraging results have been obtained in translating poliovirus and encephalomyocarditis (EMC) RNA (a related virus) *in vitro* with systems derived from their physiological host cells. J. V. Maizel *et al.* (Albert Einstein College of Medicine) have been able to isolate polysomes from poliovirus infected HeLa cells; this makes a major protein product about the size expected to correspond to the whole genome. A. E. Smith (MRC, Cambridge) reported that EMC RNA can be translated *in vitro* by a system derived from uninfected Krebs IIA cells, and has identified one of the sites where the EMC protein is cleaved.

Noticeably fewer faces were present when the meeting ended, but those whose minds had clotted earlier in the proceedings missed the first announcement of the finding of termination factors in mammalian systems. J. L. Goldstein, A. L. Beaudet and C. T. Caskey (NIH) have obtained a protein preparation, admittedly crude at present, which assists termination on reticulocyte ribosomes. In both bacterial and mammalian systems, fMet-tRNA_f is "sticky"; it binds non-enzymatically but securely to ribosomes. Goldstein *et al.* have taken advantage of this to prepare an assay for termination; the appropriate codons or messengers can be added to fMet-tRNA_f bound ribosomes and release of formyl methionine is measured. This shows that there is a factor which responds to the codon UAA in reticulocytes, and the NIH workers suspect that their preparation also contains a stimulatory factor which assists its action (a similar mechanism prevails in bacteria). No release factor responding to the other nonsense codons, UAG and UGA, has been identified yet.

Another interesting result of this work is that it has proved impossible to differentiate the action of peptidyl transferase (the ribosomal enzyme which synthesizes peptide bonds) from the release activity.

Although these two activities are inhibited by a different spectrum of antibiotics according to whether bacterial or reticulocyte ribosomes are used, in each case, any particular antibiotic inhibits either both activities or neither. This supports the idea (see *Nature*, 227, 337; 1970) that peptidyl transferase plays some part in the termination of protein synthesis.

PARASITIC FISH

Suckers and Pickers

from our Marine Vertebrate Correspondent

THE very specialized fishes known as remoras, shark suckers, or diskfishes (in the United States) have for long attracted the attention of man. Their habit of attaching themselves by the powerful sucker on the back of the head to larger fishes, especially such fearsome creatures as sharks, turtles, and even ships, always merited a passage in the earliest zoology. They made their mark on history, too, for Pliny recounts that Mark Antony lost the naval battle of Actium because at the critical moment his ship was held back by a remora. For all this long-standing interest in the habits of these fish their life history and biology has been relatively unknown. For this reason, the publication by Roger F. Cressey and Ernest A. Lachner of a paper on their diet and life history (*Copeia*, 1970, 310; 1970) is of considerable interest.

Eight species of these marine fishes are now recognized, of which specimens of six were examined in Cressey and Lachner's study. Several interesting features emerge from their data and from the literature. First, several of the more morphologically specialized species are strongly host specific. One species, *Remora australis*, has only been found attached to whales and dolphins; another, *R. osteochir*, is almost always found on swordfish and their allies; while *Remorina albescentis* is usually found in the mouth or gill cavity of the giant tropical devil fishes or mantas.

The association between remora and host may not be so casual as had for a long time been thought, for it has often been claimed that the association was ephemeral. But Cressey and Lachner suggest that a single host may be the life abode for the remora, and they report finding entire "families" consisting of small juveniles, subadults, ripe males and gravid females on the same host. Although conclusive evidence is lacking, the authors feel that it is likely that mating or other reproductive behaviour takes place while the remoras are attached to the host. The very earliest young stages are, however, known to be free swimming. An indication that supports their suggestion that the association is more permanent than had been thought previously lies in the relative swimming abilities of the hosts and the species of remora. Observations on living fish show that they cannot match the speed or endurance of most of the host species (which include some of the most powerful swimmers known). For this reason alone it seems unlikely that the remora would frequently completely detach itself, for once out of touch it would probably not catch up again!

Cressey and Lachner's primary contribution to knowledge of these fish, however, lies in their detailed confirmation that remoras act as cleaners to their hosts, often feeding primarily on the external parasites of the body and branchial regions. This had first been

suggested by two Argentinian ichthyologists, Szidat and Nani, as long ago as 1951, and later observations by other workers tended to confirm their suggestion. These observations were, however, based on very few fish examined, but the present study of the stomach contents of 401 individuals shows that the habit is widespread in the group. In only one of the six species investigated was the evidence inconclusive. The role of parasite-picker varies with the species involved: the commonest species, *Remora remora*, is an active cleaner, and the mutualistic relationship between fish and host is strong, but the mutualism is less strong in other species. Parasite-picking varies with the size of the individual although not consistently through the group—some are more active when young than when adult, some adopt the habit with maturity, while *Echeneis naucrates* is active as a cleaner in middle age.

BEES

Queens and their Consorts

from a Correspondent

A QUEEN honeybee lays fertilized eggs in worker cells in the bee comb but unfertilized eggs in the slightly larger drone cells. How does she differentiate between them? At the fifth conference of the Bee Research Association held at Grantley Hall in Yorkshire from August 21 to 23, Dr N. Koeniger (Bee Research Institute, University of Frankfurt) reported noticing that when the queen inspects a cell before laying in it, she inserts not only her head into the cell, but also her front legs. By attaching "spurs" of sellotape to a queen's forelegs, Dr Koeniger made it impossible for her to get these legs into a cell during inspection. All the eggs the queen then laid were fertilized, whatever the type of cell, but when the sellotape "spurs" were removed, the normal pattern of laying was resumed. These experiments and others, in which various parts of the forelegs were amputated, show that the queen uses her forelegs in some way to differentiate between the two types of cell, but the mechanism remains a mystery.

Worker bees—infertile female members of the colony—carry out most of the household duties in the hive, such as comb building, food gathering, cleaning of the hive, feeding the queen and the developing brood, and so on. They also seem to control the queen's egg laying to a large extent according to Dr Delia Seager (University of Aberdeen). Dr Seager described how, when a colony is preparing to swarm, egg laying is reduced and the "retinue" of workers stops feeding and grooming the queen. Instead, the workers shake the queen constantly and quite violently, sometimes several hundred times in an hour. The result of all this treatment is that the queen is slim and muscular enough to swarm.

With the distinction between unfertilized and fertilized eggs being so important in the social structure of the bee colony, attention is now turning to the largely unknown mechanism of fertilization. Dr G. Koeniger (Bee Research Institute, University of Frankfurt) described a new technique which throws some light on the process. After two years' experiments she has perfected a method which involves making an incision in an abdominal tergite of the queen (avoiding the flexible intersegmental membrane), lifting a small

flap to give access to the internal organs and replacing the flap, all within 10 to 15 minutes. The queen immediately licks the wound, which heals, and she seems to live a normal life in the colony for several weeks. Using this method, Dr Koeniger has succeeded in removing the spermathecal gland from living queens, who, after the operation, return to their colonies and resume egg laying. When both branches of the gland are removed, only unfertilized (drone) eggs are laid afterwards. In contrast, when only one branch is removed, the queen lays worker and drone eggs normally in the appropriate cells, indicating that the operation itself does not cause the change. It seems therefore that the spermathecal gland must be involved in releasing from the spermathecae the spermatozoa which fertilize the female (worker) eggs.

PLANETS

Primaevial Surface of Mars

by our Astronomy Correspondent

THE latest chart of Mars, published in August by the US Army Topographic Command, is something of a disappointment. For the most part it looks like charts of Mars always have done—a shadowy patchwork of light and dark areas with science fiction names like Panchaia, Memnonia and Xanthe. Two patches showing greater detail in the shape of fuzzily drawn craters hardly do justice to the original Mariner photographs.

It is now more than a year since the close approach of Mariners 6 and 7 to Mars. As with the early Moon probes, the Mariners have revealed some fascinating new features on the surface of Mars but have failed to answer the basic questions that are in everyone's minds. Coinciding with the publication of the new Mars chart, Commission 16 of the IAU dealing with planets and satellites met last week to review the new data.

Electronic processing of the more than two hundred television pictures has provided full-time work for about a dozen people. Electronic noise has been removed from the pictures, they have been corrected for geometric distortion, and enhanced versions to bring out the faint features have been produced in the computers at Caltech.

Even the depth of the icecaps, at one time thought to be a sprinkling of carbon dioxide snow, is now in question. Near-encounter pictures of the south polar region show what are being called "etch pits" apparently tens of metres deep that seem to be in the snow rather than the underlying topography. If the snow is as deep as this, it is hard to see how all of it can be removed during the seasonal cycle. So the Mars mission in 1971 will be on the lookout for quasi-permanent drifts of snow that have escaped attention, possibly because of a covering of dirt.

More than anything else, the surface of Mars seems to resemble a worn-down version of the lunar highlands. The highlands-maria dichotomy is absent, however, and the line being taken by the Mariner Television Experiment Team is that what is being seen is the basic accretion surface. But there are two areas which suggest that internal processes may be beginning on Mars. One is a featureless area in Hellas with no craters down to the 300 metre resolution limit, indicating that only a comparatively short period of time is

taken by the process that wipes the surface clean. The second possible signature of underground processes is a patch of what is termed "chaotic terrain", an almost craterless area made up of short ridges and depressions typically 1 to 3 km wide and 2 to 20 km long.

More has been learned about the atmosphere, however. The television experiment detected a multilayer structure at the limb, from about 10 km up to 50 km, which the infrared spectrometer experiment indicates may be solid carbon dioxide. Dr G. Pimentel reported that there is also infrared evidence for silicate dust in the atmosphere, and a "quite low" abundance of H_2O may have been detected. New upper limits are being placed on the extent of NO , N_2O , OCS , CH_4 , O_3 and NH_3 . As expected, the ultraviolet spectrometer shows a spectrum similar to the laboratory spectrum of carbon dioxide irradiated by sunlight, but what is odd is the scarcity of atomic oxygen. Carbon dioxide in the laboratory is easily broken down by sunlight. A broad absorption band at 2500 Å interpreted as ozone is detected over the pole caps, but not over the unfrosted areas. Either the pole caps are acting as a cold trap, or for some reason the band is easier to see against a light background. The ultraviolet has also shown that aerosols of sizes less than a tenth of a micron or so are present in large quantities, scattering twice as much light as the atmosphere itself.

It is pleasing that the different methods of drawing the elevation profile of the Martian surface—by radar from the Earth, and by measuring the depth of atmosphere at different points from the intensity of spectral lines—are more or less in agreement in areas where they have all been tried. But the elevation differences that have been measured, possibly as much as 15 km between highest and lowest points, are comparable with the interval between the deepest ocean and the highest mountains on Earth and raise the question whether isostasy is also at work on Mars.

PALAEOMAGNETISM

No Correlations in the Canaries

from our Geomagnetism Correspondent

ONE of the most fascinating unsolved problems in palaeomagnetism is the source of the correlations between magnetic polarity and oxidation state found in rocks from certain sites. The construction of a polarity-time scale for the past 4.5 million years, the deduction from magnetic anomalies at sea that alternating periods of normal and reversed polarity extend backwards in time for many tens of millions of years, the evidence from baked contact rocks and the fact that about fifty per cent of the world's rocks are reversely magnetized, leave no doubt that almost all reversed rocks were produced during periods when the Earth's magnetic field was reversed. This being the case, there should be no correlation whatsoever between the magnetic polarity of rocks and their physical, chemical or mineralogical properties. Indeed, the existence of such correlations would be positive evidence for self-reversal, a phenomenon known to exist but support for which is overwhelmed by the evidence in favour of field reversal.

What, then, can be the explanation for the observed correlations? Reversed Quaternary and Tertiary lava flows from Iceland, reversed Tertiary flows from Japan

and reversed Carboniferous flows from Scotland all contain significantly more separate ilmenite than the normal flows from those sites; and statistically, the titanomagnetites in reversed Tertiary flows in Scotland, Iceland and the Columbia Plateau, Oregon, and in reversed dykes in Scotland are more highly oxidized than those in the corresponding normal rocks. The first question which needs to be asked is, of course, whether these correlations are fortuitous. After all, they represent a negligible proportion of the world's rocks, and many of these particular samples obey the classic tests for field reversal. The reason why so few correlations have been reported is simply that the work involved is long and tedious. Yet in spite of the small number, they share one property which argues against their arising by coincidence—they are all in the same sense. It is always the reversely magnetized rocks which are, on average, more highly oxidized, never the normal ones.

Even so, the significance of the oxidation-polarity correlations will ultimately be firmly established or rejected by statistics; and that is why a new report by Ade-Hall and Watkins (*Geophys. J.*, **19**, 351; 1970) is important. For these authors have carried out a petrographic and magnetic analysis of 168 Miocene to Pliocene basalt, ankaramite and phonolite lava flows from the Canary Islands, and find no correlation whatsoever between polarity and oxidation. There is, of course, always the possibility that the correlation really exists but is obscured in some way; but Ade-Hall and Watkins have carefully considered this problem and conclude that it does not arise here. It is possible, for example, that a collection which comprises three different rock types, each of which possesses a different but real polarity-oxidation relationship, might exhibit no overall correlation. A separate analysis for each rock type represented, however, revealed a correlation for none. Then again, it is possible that a correlation could be obscured by experimental limitations. It may, have been, for example, that the $\times 1200$ magnification used by Ade-Hall and Watkins in their petrographic analysis was insufficient to resolve fine exsolution of ilmenite in the titanomagnetite grains. This was also ruled out by low temperature Curie point measurements which failed to indicate the presence of any ilmenite at all.

So how does this new non-correlation affect the status of the oxidation-polarity problem in general? The answer is that by itself it makes little difference to the overall situation, for one brick is sufficient neither to build nor to demolish a structure. But it does have a two-fold importance. For one thing, it emphasizes, together with two other non-correlations, that oxidation-polarity relationships are not universal. This in turn serves to emphasize the statistical nature of the whole problem. And second, it stresses that, in this matter, negative results—the absence of correlation—are as significant as positive data. The long-term problem is, of course, that a vast amount of work will be necessary on other rock collections before it can be proved that the correlations observed hitherto are not trivial. But if the case is ultimately proved, it will be a major headache for Earth scientists. For, given the reality of field reversal, it is difficult at present to imagine what physical connexion there could be between the oxidation state of lavas which originate in the upper mantle and the magnetic field which is produced in the Earth's core.

COSMIC RAYS

Backing for Muon Theory

A NEW analysis of the spectrum of muons from cosmic rays at energies of more than 1,000 GeV has given added support to the notion that muons may be produced by way of a special "X process" involving a short lived intermediate particle. This idea was first put forward by H. E. Bergeson *et al.* (*Phys. Rev. Lett.*, **19**, 1487; 1967) to explain the unusual angular distribution of muons from cosmic rays detected in an experiment in Utah.

Bergeson *et al.* contended that the measured angular distribution was not consistent with the muons' being solely the byproduct of pions and kaons produced in the upper atmosphere. Some other process had to be invoked, and they suggested that the solution lay in what they called an X process, for which the variation with energy of muon production was flatter than for production from pions and kaons. One interesting possibility was that the X was in fact the intermediate boson, which is held to mediate the weak interaction but has so far eluded detection. Another view was that an integral charge SU3 triplet was responsible for the X process.

Bergeson *et al.* pointed out that the spectrum derived from a combination of muons from pions and kaons with those from the X process give—with one exception—a convincing fit to other cosmic ray data between 10^{11} and 10^{14} eV, and could also account for the existence of mu-less air showers at 10^{15} eV. There has, however, been some opposition to this scheme, given fuel by the need to assume that the photonuclear cross-section increases at energies of more than 1,000 GeV to five times the value that it has at normal accelerator energies, about 20 GeV.

P. Kiraly and A. W. Wolfendale (*Phys. Lett.*, **32**, 510; 1970) have shown that the presence of an X process need not depend on a large rise in cross-section. The weak link in the reasoning, they suggest, lies in the fact that the Osborne-Wolfendale-Palmer spectrum, which was the previously accepted muon scheme, was not measured directly by Bergeson *et al.* in this energy range but rested on the correlation of vertical cosmic ray intensity with depth underground and an assumed rate of energy loss. Kiraly and Wolfendale claim that if a higher rate of energy loss is taken the need for such a large cross-section is removed.

In the original analysis the X particles were assumed to have the same form of production spectrum as the pions and the vertical muon spectrum was derived from the angular distribution of the data. Kiraly and Wolfendale have followed recent practice by taking a larger constant in the energy loss formula. They also used the evidence from gamma ray experiments of a lower limit to the number of muons at sea level produced from pions. By then making the reasonable assumption that the number of muons produced by the X process is about 1.5 per cent of that from pions they show that the number of muons from pions at sea level can be made consistent with the evidence from gamma rays. This means that a big increase in photonuclear cross-section need not be a precondition of the existence of the X process. They also point out that the assumption of Bergeson *et al.* that the pion spectrum has the same slope as the primary spectrum is not necessarily true.

MATERIALS

Special Ceramics

from a Correspondent

RAPID advances in modern technology, for example in the nuclear power and gas turbine industries, are creating increasing demands for special materials with exceptional properties. Ceramists are turning to silicon nitride, silicon carbide and alumina, in that order, according to Dr N. F. Astbury, director of the British Ceramic Research Association, who gave the opening address at the Fifth International Symposium on Special Ceramics held in Stoke-on-Trent from July 14 to 16. Other papers at the conference dealt with pyrolytic materials, the fabrication of ceramic bodies and the determination of the physical and chemical properties of a wide range of materials.

E. Voice (Atomic Energy Establishment, Winfrith) described the deposition of pyrolytic silicon carbide from methyltrichlorosilane-hydrogen mixtures in a fluidized bed of carbon-coated nuclear fuel particles. He showed that the temperature and gas composition are critical for the production of a fully dense impermeable layer. The morphological aspects of such layers were discussed by D. E. Y. Walker (UK Atomic Energy Authority, Springfield Works), and J. Ashford and J. C. Hodgson (Central Electricity Generating Board, Berkeley) described a technique for determining the mechanical behaviour of the layers under static and cyclic stress.

Because of the difference in thermal expansion it has been impossible to obtain strong vacuum tight metal-silica seals without using "graded silica-glass" components. L. Verheyden, K. Klein and H. Kind (Euratom, Italy) showed that silica can now be joined directly to 'Kovar' (54 per cent Fe: 28 per cent Ni: 18 per cent Co) by using a composite wire with a core of titanium surrounded by a 72 per cent Ag: 28 per cent Cu alloy.

The fourth and final session of the symposium was devoted solely to silicon nitride ceramics. P. Grieveson with K. H. Jack and S. Wild (University of Newcastle upon Tyne) described techniques whereby, at a given temperature, the nitrogen and oxygen partial pressure required to form silicon nitrides and oxynitrides is accurately determined, showing that α and β are not low- and high-temperature forms but are "high oxygen potential" and "low oxygen potential" modifications. D. J. Godfrey working with M. W. Lindley (Admiralty Materials Laboratory, Poole) showed that reinforcement with carbon fibres or tungsten wire improves the toughness of silicon nitride tubes and increases the fracture stress. A novel technique for producing cellular silicon nitride was described by T. W. Lindop (Doulton Industrial Products). This low density material has many potential applications in the nuclear reactor and refractory industries. The results of an investigation by S. Wild, P. Grieveson and K. H. Jack (University of Newcastle upon Tyne) and N. J. Latimer (The Plessey Company) on the effect of magnesia additions on the hot-pressing behaviour of silicon nitride account for a number of previously unexplained features. At high temperatures, a liquid of composition near to that of enstatite (MgSiO_3) is formed and this acts during pressing as a flux and plasticizing agent to allow complete densification of the compact.

British Association—Durham, 1970

What is it all for?

WITH the cricket season drawing towards its close and with migratory birds about to make preparations for moving south, it is only natural that the British Association should once more be assembled, this time at Durham. There is no reason why the meeting should not be a modest success—the programme has been still further honed down, the sections seem to have been even more selective than in the past few years, while the North of England is known to be a generous supporter of all kinds of improving causes, the British Association among them. Unhappily, however, the association as a whole seems still to be divided within itself about its function in the modern world. Is it a scientific society or is it a public debating society?

The days are long since gone when a general interest in science could sustain and spur on the development of the several disciplines to which the British Association still devotes a section of its activity, yet there is evidently a less original but nevertheless important role to perform in carrying not merely news of new developments but a sense of their importance to the growing community of people with a professional interest in science, not necessarily in research. So much is beyond dispute, although the association still has some way to go before it can be thought to perform that task as expertly as circumstances require. So should the association set up also as a debating society? For one thing, there is no necessary inconsistency between such a function and its more traditional role as a purveyor of enlightenment. Indeed, there are many points at which the two interests should properly work together. Is there not, for example, a case for thinking that problems such as the proper siting of power stations or the uses to be made of supersonic air transports would be more easily resolved if there were a forum in which the facts and people's expectations of them could more directly be brought into confrontation with each other. In short there is room for a responsible debating society, and of course there is no reason why such a forum should become the kind of proceeding that turned the meeting of the American Association for the Advancement of Science, at Boston last Christmas, into what seems to have been a mixture of pantomime and student demonstration.

Not the least of the issues of public policy that needs to be taken up is the condition of scientific research itself. The pages which follow are designed so as to draw attention to some of the fields in which important and exciting developments have taken place in the past year. The number of topics could have been multiplied by two or three—there is nothing, for example, in what follows about the exciting developments which have kept astronomers on their toes for the best part of a decade. In the same way, the most recent and most fashionable of all the developments in molecular biology in the past few years—the demonstration of how the nucleic acid in RNA viruses can be used as a template for the manufacture of characteristic DNA—has been omitted from this survey, if only because the pages of *Nature* have been full of it for

weeks. And then the survey also neglects the important developments in those branches of physical science that are continually making a more intimate connexion between solid state physics and electronics, laser technology for example, the solid but important work on the borders of genetics and descriptive zoology that promises to throw an important new light on the dynamics of populations and indeed the whole concept of the balance of nature and the ways in which the craft of science is at last making its mark on the somewhat intangible problems that preoccupy the social scientists. The pages which follow describe, in short, the tip of an iceberg. Moreover, they show that the condition of science is—for the time being at least—as good as anybody could hope. This is why it is important to ask urgent questions about the steps that must be taken to ensure that scientific research remains in good health. The British Association should not shrink from playing its part in that important task. Not the least of the contributions it could make would be to demonstrate the importance of the role that scientific research continues to play in the modern world.

The most obvious threat to the condition of science is the prospect that funds for scientific research will not grow as quickly in the years ahead as the numbers of people leaving postgraduate courses at the universities and anxious to stay on in research. This is where most anguish has been caused in the past few months, in the United States especially. It is therefore important to recognize that the link between the scale of support and the pace of discovery is not nearly as simple as it sometimes seems. Doubling the amount of money for research on tumour viruses now that it seems as if there may be a few tangible points at which careful investigation may provide a route to the better understanding of some kinds of cancer will not, by itself, accelerate the pace of discovery by a factor of two or anything like it. On the other hand, there is a point at which the lack of money or other forms of support may seriously hamper the efforts of individual researchers, if only by distracting them from serious work. But the more important criterion for deciding the scale on which financial support for research is needed is bound up with poorly understood matters concerning the proper balance of the scientific community as a whole.

Thus there is a case for thinking that experienced investigators are more buoyed up by their work if they are

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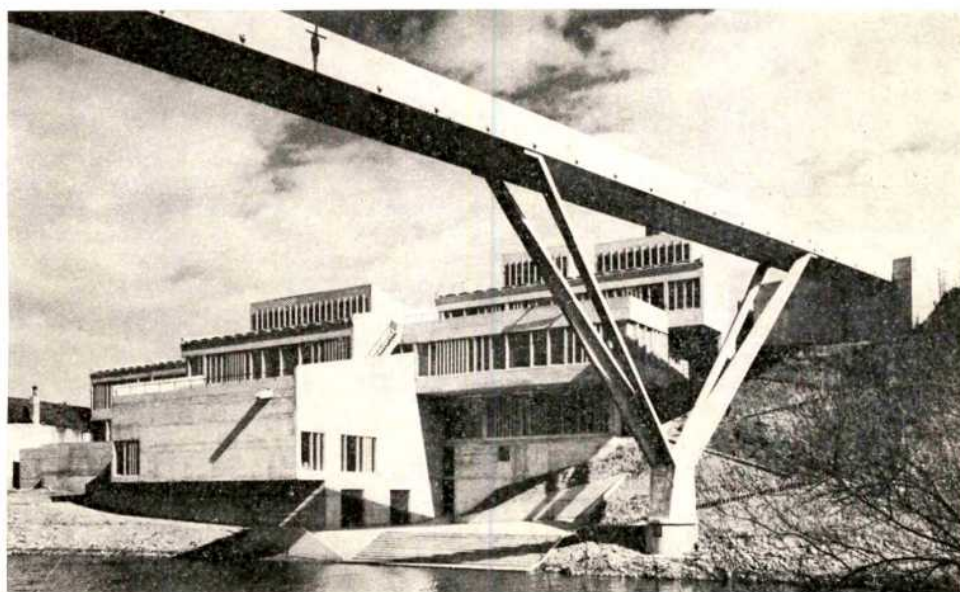
associated with younger people with whom they can share ideas and aspirations. Young people themselves seem to enjoy the competitiveness of research. And most people at all levels appear to be immensely stimulated by the connexion between research and education. It follows that a good many of the arguments about the exact amounts of money spent on scientific research at universities and elsewhere, which are the best approximations so far to what might be called a public discussion of science policy, are often well beside the point. Professor John Ziman (see p. 996) has done a great public service by drawing attention to some of the subtleties of the way in which the scientific community is organized. The first conclusion to be drawn from all this is that the capacity of the scientific community to sustain the momentum of its research is probably a good deal better than a mere counting of pennies would suggest. On the other hand, there are many ways in which a thoughtless handling of problems which seem peripheral to research as such may seriously damage the pace and the character of the original work being carried out. This is why discussions of the condition of science are inseparable from a consideration of the condition of the universities. It goes without saying that one of the most disturbing tendencies in the past few years has been that in which a good many universities in the west, and especially in the United States, seem in danger of losing the serenity from which, it seems, original work must spring.

But why should people worry if, by mishandling or misjudgement, the conditions in which original research is carried out should continue to deteriorate? This is the somewhat philistine question all too often asked by those on whom has fallen the responsibility for deciding whether to spend public funds on scientific research or some other public activity. In some sense, this is an issue that each person must settle for himself, and it is quite proper to ask whether the modern world would be substantially worse off without, for example, the understanding of sea-floor spreading about which Professor Vine writes on page 1013. And on the face of things, of course, it is a matter of largely academic interest that the continents

appear to drift about on the surface of the Earth—grist for the week-end magazines but hardly anything more than that. Only a little reflexion will show, however, what a shallow view that it.

The development of geophysics in a mere ten years is now well on the way to turning the whole of geology on its head. For one thing, it is now for the first time possible to understand the origins of the forces responsible for the great episodes of mountain building. The Himalayas, for example, are a measure of the northward movement of India in the past 100 million years. The African Rift Valley is now intelligible, and although dispute remains about the exact meaning to be given to the geological features of the Middle East, there is no doubt that its outstanding characteristics have been shaped by the pushing and pulling of the great tectonic plates. In exactly the same way, the seismic zones running the length of South America have been made intelligible. It can only be a matter of time before the consequences of the new tectonics for the more practical interests of the geologists are plain for all to see. But there is more than mere profit to be won from the new development. It is already clear that the new understanding of the way in which the surface of the Earth is being continually transformed has provided a new perspective for helping to make the real world rational and understandable. Like the great achievements in the past, it will help to drive away the cobwebs.

One of the most insidious dangers in the recent past has been the way in which the test of quality in science has been confused with the test of relevance. It would be a valuable exercise for those who raise the cry that scientific research should be directed towards practical ends to ask whether their present tests would have permitted the expenditure of public funds on the work which has now led to the new tectonics. The same embarrassment would be occasioned by the question whether the rest of the work described in this symposium would have qualified. Yet there can be no doubt that all of it will contribute in important ways to the more successful adaptation of people to the natural world. If the British Association needs an issue for which to fight, this would be a place to start.



Dunelm House, University of Durham, location of the General Symposia at the British Association meeting. The building was designed by Architects Co-Partnership and received the 1967 RIBA Architecture Award for Northern England (Region I). (Photo: John Donat.)

Promising Trends in Neuroscience

by

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Investigations ranging from tissue culture to animal communication are rapidly providing deeper insights into the mechanisms of brain and behaviour.

DURING the past few years neuroscience, comprising the sciences of brain and behaviour, has been differentiating, integrating, regrouping, absorbing enthusiastic recruits from other sciences and sprouting many new conceptual growing points at all levels—molecular, cellular, neural, and behavioural.

There are indications of rapid expansion in the recent appearance of several journals and series of books with the words "brain" and "neuroscience" in their titles, and the formation of new multidisciplinary societies concerned with neuroscience. These include the Brain Research Association of the United Kingdom, the Society for Neuroscience of the United States, the European Brain and Behaviour Organization, and new speciality groups such as the International Neurochemical Society and the American Society for Neurochemistry.

I have chosen the topics for this brief review from recent work sessions, conferences and intensive study programmes—at each of the four levels mentioned—sponsored by the Neurosciences Research Program of the Massachusetts Institute of Technology, an international interuniversity organization for the stimulation of advance in neuroscience (details in ref. 1).¹

Properties of Membranes

The molecular biology of axonal and synaptic membranes is a crucial part of neurobiology². Sensitive and fast physical methods, such as polarization optics, light scattering and fluorescent probes are being applied in an effort to characterize the configurational changes of membrane molecules during excitation. Electron spin labels indicate the relative liquidity of membrane lipids, particularly those that are unsaturated, while proteins and carbohydrates confer solidity, according to McConnell³. The study of model systems of lipid bimolecular layers containing specific ion carriers has suggested a solution to a problem that has long eluded physiologists—how does the cell distinguish ions which are as similar physically and chemically as Na⁺ and K⁺ (refs. 4 and 5)? The ion carrier is pictured as an ion-specific peptide cage molecule that engulfs the cation, desolvating it as it enters, and closes by fast conformational change; then, facilitated by outwardly directed lipophilic side chains, the ion-bearing cage peptide traverses the membrane under the potential gradient and discharges the resolved ion on the cytoplasmic side of the membrane⁴. Isolation and characterization of the actual membrane carriers are difficult because of their low concentration in the neuronal membrane.

Histochemical and electron microscopic evidence has revealed glycoproteins attached to the membrane matrix. These have carbohydrate chains, which contain highly acidic sialic acid residues and extend into the intercellular space. Such glycoprotein cell coats at neuronal synaptic junctions may provide the specificity and molecular recognition by which the ontogenetically correct

neurones are synaptically linked; some investigators speculate that enzymatic changes of terminal glycosyl or fucosyl groups provide the plasticity for the consolidation of experiential information as learning^{7,8}.

Neuroplasmic Dynamics and Synapses

It is generally believed that neuroplasm is constantly synthesized in the cell body and moves as a gel down the axon (and probably also along the dendrites) at a rate of about 1 mm per day. Particular substances, for example neurosecretions—enzymes which synthesize transmitter—and vesicles are translocated differentially and much more quickly (100–2,000 mm per day)⁹. Translocation has been pictured¹⁰ as a saltatory interaction between enzyme-containing vesicles and fibrous proteins, chiefly microtubules—although neurofilaments may also be involved—with protein subunits which have been isolated and characterized chemically. The transport mechanism, probably universal in cells, is primitive and conservative evolutionarily. Chemomechanical coupling of energy may underlie this mechanism as it does contractility in muscle, cilia, flagella, and perhaps also the injection of nucleic acid into microorganisms by viruses.

Electron microscopy^{11,12} has revealed the detailed structure of synaptic junctions, which seem to be composed of a single type of material, presumably a protein still poorly characterized, which includes presynaptic dense projections (that enlarge with maturity and patency of the synapse), cleft-contained material, and a subsynaptic web. Vesicles slotted between dense projections are thought to release transmitter quantally by exocytosis through the membrane into the cleft.

Although only six substances qualify as transmitters, a dozen more may be discovered. Kandel *et al.*¹³, working with the synapses of the sea slug *Aplysia*, have shown that a given transmitter can excite some postsynaptic neurones and inhibit others, while a particular postsynaptic cell can respond to more than one transmitter. If generalization is justified, these features suggest that synaptic processing is more complex than has been supposed.

Drugs such as tranquillizers, antidepressants and psychotomimetics interact at particular synaptic sites; new compounds with highly selective actions are being synthesized for research into the mechanism of synaptic action. New drugs that have proved important clinically or pharmacologically are: 6-hydroxydopamine which selectively destroys adrenergic endings¹⁴; bicuculline¹⁵ which antagonizes the inhibitory transmitter GABA (gamma-amino butyric acid); and L-dopa (dihydroxy-phenylalanine), effective in the treatment of Parkinson's disease, which is converted into transmitter necessary for normal functioning, but is either missing or in low concentrations in Parkinson's disease.

The possibility that transmitter action is modulated or

controlled by macromolecules bound to presynaptic and postsynaptic membranes or released with transmitters into the synaptic cleft has been discussed recently¹⁶. There is particular interest in a protein called chromogranin A, which is present in the vesicles of the chromaffin cells of the adrenal medulla, and is valuable as a model for the study of transmitter metabolism, storage and release. Chromogranin—present also in the vesicles of adrenergic neurones, together with catecholamine, ATP and enzymes necessary for synthesis of noradrenaline—is thought to be extruded from varicosities and endings of sympathetic neurones. The elucidation of the function of the chromogranin protein may illuminate synaptic function further.

The nucleotide, adenosine 3',5'-phosphate (cyclic AMP), plays an important and newly discovered role as a "second messenger" in synaptic function¹⁷. The concentration of cyclic AMP in the brain can be increased forty-fold by electrical or neurohumoral stimulation¹⁸. When the receptor in the postsynaptic membrane combines with the neurotransmitter, it probably undergoes a change of configuration which is coupled allosterically with the activation of a membrane-bound enzyme, adenylyl cyclase, which converts intraneuronal ATP to cyclic AMP, and triggers off a metabolic chain of events in the postsynaptic cell. There is evidence that synaptic transmission in the cerebellum activates adenylyl cyclase, which links transmitter action with the cyclic AMP system.

McIlwain¹⁹ has pointed out that neurones contain a multiplicity of enzymes that can be induced thus, which means that their synthesis can be turned on genetically, in appropriate conditions; this large repertoire of enzymes with specific, adaptive inducibility may be as significant for brain processes (such as ontogenetic specification, adaptation, and plasticity) as is the ability to form a multiplicity of synaptic interconnections. Axelrod *et al.*²⁰ have demonstrated that the excitation of neuronal input can induce a high rate of synthesis of enzymes producing the transmitter noradrenaline. Peterson and Kernell^{21,22} have shown that presynaptic stimulation, but not excitation by antidromic invasion, of the giant neurones of abdominal ganglia isolated from the *Aplysia*, increased the postsynaptic synthesis of RNA. If RNA synthesized in these conditions generates specific proteins, a "permanent" response might thus result from synaptic excitation. Clearly there is a need to investigate gene expression, particularly adaptive enzyme induction and its association with membrane processes in neurones.

Neurogenetics and Neurogenesis

The study of neuroscience requires a simple animal capable of some degree of learning which, if systematically studied structurally, physiologically, behaviourally and genetically, could clarify basic problems in the way that microorganisms and viruses have done for molecular genetics. Several groups of investigators, including those led by Benzer, Brenner, Levinthal and Nirenberg, are studying small organisms such as the fruit fly *Drosophila*, nematodes and rotifers, which have only several hundred neurones. Many mutants have already been found, and the investigations seem to have started well towards the long term goal of correlating genetic, anatomical and behavioural parameters.

Several dozen strains of mutant mice have been developed (and the search is on for more) which, as a

result of systematic behavioural, anatomical and biochemical study, have given important information about cerebellar function, myelination and the mechanism of specification of neuronal patterns in systems such as the cerebellum and the cerebral cortex^{23,24}. For example, the excellent work done by Sidman relates the motor disorder in mutant "reeler" mice to improper positioning of neurones in the cerebellum, associated with a single gene mutation.

A second type of experiment²⁵ epitomizes the important results that are emerging from the *in vitro* study of brain tissue. Bits of cerebellum, cerebral cortex and hippocampus are dissociated into suspensions of isolated cells by the action of trypsin. When swirled in flasks of culture medium, the separated, rounded cells collide and manifest specific adhesivity and develop a histotypic organization which resembles the normal tissue from which they came. If the normal pattern of cells is to be reconstituted in this way, the age of the embryo when the tissue is dissociated and placed in culture is critical to within a day or less. Clearly, individual cell types within a particular tissue, such as cerebellum, are differentiated with respect to specific cell-cell adhesivity at precise times, so that the appropriate complex net connectivity develops serially.

This sort of experiment can give information about biochemical, immunological, morphogenetic and other parameters. For example, embryonic tissue taken from the cerebellum of a mutant mouse reeler fails to reaggregate histotypically after tryptic dissociation; the defective type of network characteristic of the cerebellar cortex of reeler is formed instead.

Cloning of brain cells, that is *in vitro* culture from a single parent cell, is opening important avenues of research. Cloned cells maintain their specific characteristics through many transplantation generations. It has been possible, for example, to show that a particular and well characterized acidic protein called 'S-100', which is specific to brain in all forms studied, derives primarily from glia rather than neurones²⁶.

Nirenberg and his colleagues^{27,28} have found that cells cloned from mouse neuroblastoma have many of the properties of differentiated neurones: they produce bioelectrically active membranes capable of propagating action potentials; respond to acetylcholine; grow processes containing microtubules and neurofilaments, and are equipped with the enzymes necessary to synthesize transmitters. This experimental design obviates the uncertainties resulting from chemical analysis of bulk neural tissue and facilitates the much-needed study of gene expression in neurones and the development of a neurochemistry and neuro-immunology of homogeneous cell types.

Retinal Physiology

New techniques for the intracellular injection of dyes and the detection of histofluorescence, together with electron microscopic and neurophysiological investigations of many vertebrate species, have characterized the five major cell types in the retina according to structure and physiology. Progress has also been made in tracing the processing of information from the time when light quanta are absorbed until the production of action waves in the optic nerve. It has emerged that information is processed in the retina without the generation of action potentials (spikes), but rather with slow potentials²⁹. Because the retina is an externalized bit of central

nervous tissue, these experiments raise the possibility that the slow-wave type of information processing is more important in the central nervous system than is commonly supposed. One of the five types of retinal cells, the amacrine cells, which lack axons, apparently participates importantly in neurophysiological processing (as in the olfactory bulb) and does so without spike generation³⁰. Adey has produced other evidence for the importance of slow-wave processes in the brain³¹.

Can Apes use Language?

Man's belief in his unique possession of linguistic capacities is being challenged by experiments with chimpanzees. Work of the Gardners³² with the chimpanzee Washoe, aged 5, and Premack³³ with Sarah, aged 8, has confounded doubts that chimpanzees can use arbitrary signs to refer to elements of the environment. They can do this if the signing does not require vocalization, for which they are poorly equipped, but instead requires manipulation, which they do well. Each chimpanzee reliably uses more than 100 signs—Washoe used the scheme known as the American Sign Language, and Sarah used plastic tokens—and each uses strings of several signs. Washoe generates "Please gimme sweet", and Sarah comprehends "If Sarah give apple Jim, Mary give cherry Sarah". Generally, their performance resembles that of 2 year old children. Even so, these achievements go far beyond anything previously demonstrated. It seems unlikely that the trainers are unwittingly cuing the correct responses. The deeper doubt is that, while chimpanzees can learn a sub-set of linguistic essentials, the full range of human semantic and syntactic powers is beyond them³⁴. Still, the capacities of these primates suggest ideas about possible stages in the evolution of language. Future physiological studies may isolate brain mechanisms of communication.

Relevance to Social Needs

Psychopharmacological experiments are beginning to yield data relevant to urban crowding as a health hazard. Mice under intense psychosocial stress caused by severe crowding produce enlarged adrenal glands with increase of adrenaline and noradrenaline and the enzymes necessary to synthesize these catecholamines²⁰. Although data obtained with animals cannot be applied directly to the human situation, it is possible that similar processes play a role in producing urban ghetto disturbances and other contemporary acute reactions to psychosocial stress.

The extensive use of various psychotomimetic substances by adolescents and young adults and the widespread addiction in suburbia to "energizing" and tranquillizing drugs pose problems that are growing with alarming rapidity. Basic and clinical research in neuroscience is needed badly to help alleviate the drug menace to society.

Violent and aggressive behaviour is an all too prevalent manifestation of social imbalance in many parts of the world. Much ethological and behavioural study has been devoted to the biological aspects of this problem. Brain stimulation, through implanted electrodes, has been applied to primates in normal conditions in the colony³⁵ and also to man in experimental conditions. Stimulation of one amygdaloid region of the limbic system may aggravate violent symptoms, while stimulation of a nearby region may tend to have a calming effect³⁶. Within the framework of acceptable medical, ethical and legal codes, clinical research is being joined with therapeutic treatment in some medical centres to investigate in humans

(the only subjects able to report emotional and subjective feelings) the neural, hormonal, biochemical and genetic mechanisms of aggressive and other aberrant behaviour.

The nervous system of the infant is susceptible to permanent injury by malnutrition and hormonal, genetic, toxic and traumatic effects³⁷. Brain damage due to nutritional deficiencies is probably much more widespread than is supposed. Postnatal psychological deprivation can cause irreversible damage³⁸. Some kinds of injury can be reversed by early treatment, and, happily, some forms of biochemical impairment (phenylketonuria, homocystinuria) can be prevented by adherence to the appropriate diet.

Clearly, neuroscience can contribute importantly to pressing social and medical problems which result directly or indirectly from harmful environmental influences and from inborn biochemical, neurological and genetic aberrations. Perhaps still more important to man's progress is the possibility of a better understanding of basic mental processes which, in the end, is responsible for the advancement of science and society.

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Second Golden Age of Molecular Biology

by

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The highlights of a year when microorganisms have proved more productive than ever for studying the genetic apparatus of cells have been the isolation and synthesis of DNA genes and the sequencing of RNA phage genes. First steps have been taken towards characterizing the enzymes which synthesize DNA and degrade RNA in bacterial cells. Protein synthesis in mammals is initiated in a way similar to bacteria; this supports the idea that the mechanisms of heredity are universal even in their details. A breakthrough in understanding the action of RNA tumour viruses has been the finding that they reverse the central dogma of molecular biology by directing synthesis of DNA.

A YEAR or so ago it was fashionable to say that the golden age of molecular biology had come to an end¹ and that research should now concentrate in other areas, such as genetic differentiation or neurobiology. But the advances of the past year have shown only too clearly that "classical" molecular biology cannot be disposed of so readily. Certainly, it is true that much of its conceptual framework is now firmly established and is unlikely to be overturned²; but after passing through a comparative plateau of discovery, bacterial protein and nucleic acid synthesis is turning out to be rather more complex than it seemed; and extension to higher organisms, and to their viruses, of the approaches used with bacteria is at last beginning to pay dividends.

Although there is now a depressing proliferation of control systems—it seems that one has only to think of a control system to find that it is used somewhere—the idea that there is a basic similarity between all organisms in at least their mechanisms of protein and nucleic acid synthesis is taking an increasing hold on the direction of research. Armed with a detailed knowledge of how proteins are synthesized in *Escherichia coli*, many research teams are looking to see whether the same processes work in more complicated organisms.

The most striking similarities are those between the various protein factors required for protein synthesis; in addition to the protein components of the ribosome itself, at least eight factors associate with it at particular stages of protein synthesis. The three factors concerned with the elongation of the growing polypeptide chain have been known for some time to fulfil closely related functions in *E. coli*, yeast, reticulocytes and rat liver. Factors to assist the termination of protein synthesis have so far been found only in *E. coli*, but there is no reason to suppose that, when found, they will prove to be much different in other organisms.

Initiation of Protein Synthesis

Despair that protein synthesis may start in different ways in different systems arose from the suggestion that N-acetyl-valine can initiate haemoglobin synthesis *in vitro*³, and that histones may start with N-acetyl-serine⁴; for in *E. coli*, protein synthesis is initiated only by N-formylmethionine. An AUG codon at the beginning of a gene codes for formylmethionyl-tRNA_i; this is a methionyl-tRNA in which the free amino group of the

amino-acid has been blocked by addition of a formyl group. *E. coli* cells also possess another type of methionine accepting tRNA (tRNA^{Met}_m), which cannot be formylated and which is used to provide methionine residues within proteins. For *E. coli* proteins which commence with methionine, the formyl group is subsequently removed from the nascent protein by a specific enzyme; and for proteins which commence with other amino-acids, the terminal methionine is also removed to reveal the proper N-terminal amino-acid⁵.

Hopes that most proteins may after all start in the same way have been revived by the finding at the MRC laboratory in Cambridge that mouse ascites tumour and liver cells contain two methionine tRNAs, one of which can be formylated *in vitro* by the *E. coli* transformylase enzyme, and the other of which cannot⁶. The formylatable species places methionine at the N-terminal end of proteins, and the non-formylatable species provides methionine for internal sites⁶. But although the two mouse methionine tRNAs seem to play roles analogous to those of *E. coli*, neither is formylated *in vivo*. The ability of the initiator Met-tRNA to be formylated may therefore be an evolutionary hangover.

Haemoglobin also seems to be initiated in a manner similar to bacterial proteins. Both the α and β chains of rabbit globin are initiated with a methionine residue which is removed during the early stages of polypeptide chain growth to reveal the physiological N-terminal amino-acid, valine⁷. As a similar mechanism initiates the synthesis of protamines (basic proteins) in trout testis cells⁸, this form of initiation may be in general use.

One problem raised by the existence of two types of methionine tRNA, each of which responds to the same codon (AUG), is how only the initiator tRNA responds to those AUG codons at the beginning of genes, but the internal coding tRNA responds within the message. In *E. coli*, the elongation factors which transfer tRNA to the ribosome recognize all normal aminoacyl-tRNAs, but do not interact with the initiator formylmethionyl-tRNA_i. Conversely, the factors involved in initiation bind only the initiator tRNA and no other aminoacyl-tRNA. Reticulocytes probably have a similar mechanism, for their initiation factors preferentially bind initiator Met-tRNA and their elongation factors discriminate against this tRNA⁹.

Ribosomes also must be able to distinguish AUG initiator codons from AUG triplets within messengers,

perhaps by recognizing some nucleotide sequence somewhat longer than the AUG codon itself. The most promising approach to resolving the dilemma of the ribosome in deciding the meaning of AUG codons has come from the sequencing of RNA phages. The f2 phages have as their genetic material a molecule of RNA some 3,500 nucleotides long which comprises three genes; these code for maturation protein, coat protein and an RNA replicase which reproduces the phage. More than 500 residues have been sequenced during the past year, including the regions at each end of the RNA molecule, the initiation regions at the beginning of each of the three genes, the termination region of the coat protein gene, and also a considerable proportion of the internal region of this gene.

The initiation regions of the three genes have been characterized by allowing ribosomes to bind to the RNA phage to form an initiation complex. Treatment with nuclease then destroys the RNA molecule, except for the initiation sequences, which are protected by the ribosome binding¹⁰. All the initiation sequences have an AUG codon, and curiously, all also have a UGA termination codon in phase a few triplets before the initiator codon. But there seems to be no extensive similarity in the sequences bound to the ribosome. This means that there may be more than one type of ribosome recognition sequence on a messenger, and this could act as a control over its translation.

The chain termination sequence of the coat protein gene contains two different terminator codons in immediate succession, UAA UAG¹¹. The general significance of this redundancy is not clear, but the proper termination of proteins may be so important that this is a precaution against the suppression of termination that takes place when certain tRNAs are mutated so as to recognize nonsense codons instead of their proper codons.

An intriguing result of the sequencing is that there seem to be large stretches of the phage that are not translated into protein. As well as regions at each end of the phage (at least the first 100 nucleotides at the 5' end, and at least the last 50 at the 3' end) which do not code for protein, there are untranslated sequences separating the genes. Phage Q β , an RNA phage unrelated to the f2 group, also has a long (175 nucleotides) untranslated 5' terminal region¹². So far as can be judged from the sequences now known, the f2 phages have an extensively ordered secondary structure with many hairpin loops. Although it may be somewhat easier to discover their functions when the complete phage sequence is known, one possible role for the untranslated regions and secondary loops may be to control the translation of the phage RNA into protein (see *Nature*, **226**, 1093; 1970). In spite of some speculation, however, there is no reason to suppose that this sort of control mechanism also applies to bacterial messengers.

Control of RNA Synthesis

Protein factors are making their presence felt in virtually every area of molecular biology. Until very recently, almost all that could be said about the mechanism of RNA transcription was that it is catalysed by the enzyme RNA polymerase. But it is now clear that although the basic enzyme possesses the catalytic activity which synthesizes phosphodiester bonds, the initiation and termination of transcription are assisted by protein factors. The precise role which the termination factor, rho¹³, plays *in vivo* is muddled at present, for *in vitro*,

at any rate, there is more than one way in which RNA synthesis can be terminated (see *Nature*, **226**, 1093; 1970).

The RNA polymerase of *E. coli* is a multimeric protein, one subunit of which, the sigma factor, is concerned with the initiation of RNA synthesis. The sigma factor is released from the RNA polymerase-DNA complex as soon as synthesis of the RNA chain has begun, leaving the task of transcribing DNA into RNA to the remaining subunit complex, the core enzyme². It is the sigma factor which determines the specificity of transcription; the core enzyme by itself can use DNA as template for the transcription of RNA, but does so by transcribing only random segments of the genome. When the sigma factor is present, by contrast, transcription takes place only from certain starting points (promoters)¹⁴.

But the role of sigma extends beyond merely restricting the core enzyme to transcribing certain bacterial genes, for more than one such factor has been identified. It is true that there is only one sigma factor in bacteria, but the production of further such factors plays an important role in bacteriophage infection. When a T4 bacteriophage infects an *E. coli* cell, only certain parts of its genome (the immediate early genes) are transcribed. This is because the *E. coli* sigma factor recognizes only their promoters¹⁵.

One of the first proteins synthesized after phage infection is another sigma-like factor, which displaces the bacterial factor and directs the core enzyme of the host cell to synthesize RNA from a different set of bacteriophage genes (the delayed early genes)¹⁶. During infection, some of the subunits of the host enzyme are modified; although this does not affect which early genes are transcribed, one function of this modification may be to change the affinity of the core enzyme for sigma-like factors, so that later in infection it associates with a second phage-coded sigma-like factor; the production of this sigma factor results in transcription of another set of bacteriophage genes (the late genes) (see *Nature*, **226**, 1093; 1970).

Killer Ribosomes

Bacterial messenger RNAs are notoriously unstable, with half lives of a few minutes only. But although a good deal is known about the synthesis and translation of mRNA, it has proved more difficult to characterize the enzyme(s) which must be responsible for its degradation. Almost all that is known for sure about messenger breakdown is that the degradation of a messenger seems to follow, as it were, the tightly packed cluster of ribosomes which translates the message. As the ribosomes themselves follow closely after the RNA polymerase which is synthesizing the messenger, this means that degradation at one end begins even before the far end has been synthesized².

Many ribonucleases have been found in *E. coli*, but that responsible for degrading messengers has remained remarkably elusive. The fifth ribonuclease to be found (ribonuclease V), however, has at least some of the required properties¹⁷. This enzyme seems to be part of the ribosome and degrades mRNA from 5' to 3' in a sequential manner; it is specific for mRNA and does not attack rRNA. The enzyme activity requires all the components necessary for the movement of ribosomes along messengers and is inhibited by inhibitors of protein synthesis. This may mean that some fixed proportion of ribosomes contains this enzyme, and when one of these "killer" ribosomes attaches to the message, it degrades

the mRNA as it moves along at the end of the cluster of ribosomes.

But the isolation of what may be a mutant in a messenger-endonuclease lends credence to the idea that the degradation of mRNA may not always be as simple a process as the molecular biologist would hope. The existence of this enzyme has been inferred from the finding of a mutant which relieves polarity in *E. coli*. Polarity is the term coined to describe the observation that a nonsense mutant in one gene of a bacterial operon may have two effects; as well as inactivating the protein coded by the gene in which it occurs, it may also cause a reduction in the amount of (normal) protein(s) synthesized by genes subsequent to that mutated². Although polarity is at least partly caused by the reduced translation of the portions of the polycistronic messenger beyond the nonsense codon (presumably because the ribosome dissociates from the message at the nonsense site), transcription may also be affected. The messenger representing the operon in such cases is smaller than usual because it lacks the segment corresponding to the part of the operon beyond the mutant site. Two explanations have been proposed to account for this; transcription as well as translation may for some reason come to an untimely end at the nonsense codon so that the distant part of the messenger is never synthesized; or this part of the messenger may be synthesized, but is broken down so fast by nuclease action that it is not available for translation.

The distant part of the messenger might be defenceless before the onslaught of the nucleases because the ribosomes which would protect it from such an attack have dissociated from it at the nonsense mutant site. This theory would require an endonuclease to be involved in messenger degradation, because the first section of the mRNA would be protected by the ribosomes translating it. Support for this idea comes from the finding of a mutant of *E. coli* in which polarity is generally relieved; in the tryptophan operon, at least, this relief is associated with the reappearance of the messenger corresponding to the distant parts of the message¹⁸. This could be explained if the mutant had an inactive messenger-endonuclease. After the message had been cleaved internally by the endonuclease, a second enzyme could degrade the fragments released; this enzyme would not need to work in the 5' to 3' direction, but could perhaps work backwards from the site of cleavage in a 3' to 5' direction.

Elusive Replicating Enzymes

The most central issue of molecular biology is perhaps that of how the genetic material is replicated. A good deal is known about the mode of DNA replication²; it is semi-conservative (probably taking place by unwinding of the two parental strands, each of which then synthesizes a complementary strand by base pairing); the accuracy of the process does not depend entirely on the specificity of hydrogen bonding between base pairs but is to some extent influenced by the replicating enzyme(s); it is discontinuous, each of the new DNA strands being made in short fragments which are only subsequently covalently linked together.

But although some of the ancillary enzymes and genes involved in replication have been identified (such as the polynucleotide ligase which joins together the discontinuous segments), the enzyme(s) which actually replicate the DNA remain elusive. The Kornberg DNA polymerase, long hailed as the replicating enzyme, will, it is

true, replicate DNA *in vitro*, but the manner in which it does so does not support the idea that it replicates DNA *in vivo*. This enzyme prefers single stranded DNA as template, and does not work well when duplex DNA is its substrate.

Suspensions that the Kornberg enzyme may be concerned with the repair of damaged DNA in the cell and not with replication have hardened with the characterization of the many catalytic activities which it can undertake *in vitro*¹⁹, and with the isolation of a mutant of *E. coli* which lacks the enzyme activity when assayed *in vitro*. DNA polymerase can not only synthesize DNA *in vitro*, but can also hydrolyse the strands of a DNA duplex in either direction, or can instead use pyrophosphate ions to achieve pyrophosphorolysis in a 5' to 3' direction. Even more significant, DNA polymerase can excise thymine dimers from DNA *in vitro* (the covalent linking of adjacent thymines into a dimer is the major result of ultraviolet irradiation of cells) by releasing them into the cell supernatant in oligonucleotide fragments²⁰. Clearly, these activities would be useful for an enzyme with the cellular function of removing and replacing damaged parts of DNA, but seem less necessary for one concerned only with the replication of DNA.

But the demonstration that DNA polymerase has some function *in vitro* is no longer convincing as evidence that it does so *in vivo*. A surer guide would be to see what cellular properties are impaired by a mutation in the gene for DNA polymerase. Such a mutant was isolated by assaying many mutagenized clones of *E. coli* for DNA polymerase activity *in vitro* until one proved defective²¹; in spite of its defect, the Cairns mutant can grow normally and replicate its DNA. But it is five times more sensitive to ultraviolet light than is its parent strain, supporting the idea that *in vivo* DNA polymerase is concerned with the repair of damaged DNA²². It is not yet clear just which enzyme functions are impaired in the mutant, for it retains a normal ability to excise thymine dimers from its DNA²³. After ultraviolet irradiation, however, the mutant degrades its DNA to a greater extent than does the wild type, in a way which suggests that this degradation is due to an exonuclease. The mutant may have a DNA polymerase, therefore, which although unable to synthesize DNA can still degrade it, but in which this latter activity has somehow been altered.

If DNA polymerase does not replicate DNA, what does? The identification of other enzyme systems *in vitro* has proved difficult because the activity of DNA polymerase obscures any other DNA synthesizing activities. But two systems which lack DNA polymerase activity and can nevertheless replicate DNA have now been developed at the Max Planck Institute in Tübingen. One preparation uses a procedure in which DNA polymerase can be washed out of the preparation²⁴, and the other has made use of the Cairns mutant defective in the enzyme²⁵. Both comprise membrane-protein fractions of *E. coli* which can replicate DNA semi-conservatively at a rate comparable to that prevailing *in vivo* (DNA polymerase works only much more slowly).

The idea that DNA replication may take place at a complex associated with the membrane of the cell first arose from evidence that the origin (where replication starts) and the growing point (the present location of the replicating enzyme(s)) of bacterial chromosomes seem to be associated with the cell membrane². One possibility is that replication may not be catalysed by a single enzyme, such as DNA polymerase, but may instead

take place at a replicating complex consisting of many enzymes and associated with the cell wall (much as protein synthesis is accomplished by the enzyme complex comprising the ribosome). The task of identifying individual replicating enzyme activities may prove to be very difficult if it turns out that the complex (if it exists) can function only as a whole, and cannot be analysed enzyme by enzyme.

Isolation and Synthesis of Genes

Although the spectre of genetic engineering was raised by the isolation and synthesis of specific genes, neither of these events really marked much advance towards the prospect of new genes for old humans. The isolation by Beckwith's group at Harvard of the pure DNA carrying the genes for much of the lactose operon of *E. coli* is notable for the elegance of the experimental technique²⁶. Essentially, this involved adding the bacterial genes to the DNA of two bacteriophages in such a way that when the corresponding single strands of the duplex DNA of the two phages were prepared, each strand carried one of the two strands of the DNA of the lactose operon. The bacteriophage strands were therefore complementary in the sequence for the lactose genes only, so that hybridization of the two sets of strands gave duplex DNA corresponding only to the bacterial operon.

The isolation of much of the lactose operon in pure form, including two of its control elements, the operator and the promoter, will be invaluable in studying the nature of the interaction between proteins (such as repressors or RNA polymerase) and DNA. How a protein can recognize a sequence of base pairs in DNA remains one of the main unsolved puzzles of molecular biology. How the activity of the lactose operon is controlled is also turning out to be rather more complicated than anyone had suspected until very recently (see *Nature*, **226**, 1093; 1970), and a defined system to work with cannot be but useful.

But this type of approach is limited by its very nature to bacterial systems, where a bacteriophage may pick up a small and specified segment of the genetic material of its bacterial host. In lieu of any comparable system in the cells of higher organisms, there would seem to be no prospect whatsoever of extending a similar approach to them.

Khorana's synthesis at Wisconsin of the gene which codes for yeast alanine tRNA²⁷ is perhaps as much of symbolic as of immediate practical value. The properties of the product transcribed from this gene will certainly yield some interesting information about transfer RNA, but this is not the only way to study the synthesis of tRNA. Certainly, the synthesis of a DNA sequence corresponding to a tRNA is not in itself as important as will be the future extension of the techniques which Khorana has pioneered to the synthesis of other genes, and perhaps to their linking together into polygenic pieces of DNA.

Making a gene which codes for a protein may prove to be more difficult; even the smaller proteins would demand rather larger genes—insulin, for example, would have to be coded by a gene some twice the size of that for alanine tRNA. And the ready transcription and translation of such genes is by no means assured until the nucleotide sequences of the signals for starting and stopping transcription and translation are known. The idea that the day will soon come when extra pieces of DNA can be synthesized and added at will to the genome

of higher organisms to repair their defects is not only naive but also wrong-headed.

Dogmas and Heresies

The reverence with which DNA is regarded as the repository of genetic information in the nucleus has come under some attack in recent months. First came the proposal that DNA is present in the cytoplasm of cells of higher organisms²⁸, where it might fulfil functions analogous to those of messenger RNA. But this "information"-DNA has now fallen by the wayside, for further experiments have shown its presence in cytoplasmic extracts to be an artefact of the preparative procedures used²⁹.

The abrogation to RNA of some of the properties previously reserved for DNA has, however, proved rather more successful. The fastest rolling bandwagon ever seen even in molecular biology must surely be exploitation of the discovery that RNA tumour viruses can act as templates for the synthesis of DNA^{30,31}. Perhaps the winner of this race—if there is to be a winner—will entertain us with a counterpart to Watson's exposé in *The Double Helix*.

The RNA-dependent DNA polymerase contained in the virion of RNA tumour viruses seems to catalyse the synthesis of an RNA-DNA hybrid, using the RNA genome of the virus as template for the synthesis of a complementary strand of DNA^{32,33}. So far, the extent of hybrid formation has been shown to correspond to only a small part of the viral genome, but even so, it seems probable that hybrid formation serves as a first step towards the synthesis of a duplex DNA, corresponding to at least some of the viral genes, which can be integrated into the genome of the host cell. Other enzymes would be needed to complete this process; the virion also contains a DNA polymerase which can replicate duplex DNA and may be one of them³⁴.

It is too early to say whether this mechanism has evolved especially to enable the genetic information coded by RNA tumour viruses to be integrated into the cell genome, or whether similar information retrieval systems exist in uninfected animal cells. If there is no counterpart in the host cell to these viral enzymes, it should prove possible to inhibit selectively the action of the virus without harming the host cell.

The ease with which this discovery has been fitted into the existing conceptual framework of molecular biology shows just how little it has really upset present ideas. There is, after all, a ready way for DNA sequences to be derived from RNA. The synthesis of DNA from RNA templates will doubtless prove to be more useful for study of the unusual enzyme systems which reverse the normal relationship between template and product in nucleic acid synthesis than it will for the demolition of the central dogma.

The central dogma, enunciated by Crick, the high priest of molecular biology, has long been taken to say that the flow of genetic information from DNA through RNA to protein is a one way process which cannot be reversed. Certainly, in formal terms this idea is no longer valid. But this is of little import in more practical terms. The ultimate heresy—which would require a drastic rethinking of the whole conceptual basis of molecular biology—would be the retrieval of genetic information from protein to nucleic acid³⁵. But of this there is no sign yet.

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The Geophysical Year

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During the past year the results of deep sea drilling and other geophysical investigations have continued to strengthen the concepts of sea floor spreading and plate tectonics. Samples of lunar rock, too, have focused attention again on the history of the Earth.

1969-70 has been yet another year of spectacular advance and rejuvenation within the Earth sciences, although with the advent of lunar exploration and its associated scientific programme the term Earth sciences is something of a misnomer. The profession is buoyed up not only by the return of rock samples from the Moon and the ability to carry out physical experiments and observations on the lunar surface, but also by a plethora of results from the successful deep sea drilling programme, and the insight being gained into all aspects of geology through the pursuit of the unified concepts of sea floor spreading, plate tectonics and continental drift.

The concept of sea floor spreading was essentially a phenomenon of the 1960s; formulated by the late H. H. Hess of Princeton in 1960 and finally confirmed beyond reasonable doubt by the results of the first phase of the JOIDES deep sea drilling project in 1969. This single, revolutionary and elegant hypothesis simultaneously revived the older, but largely rejected, concept of continental drift and paved the way for the more modern and even more potent concept of plate tectonics.

Briefly, Hess¹ postulated that, as a result of convection in the Earth's mantle, the deep ocean basins are young and ephemeral features of the Earth's surface, constantly being regenerated at mid-ocean ridge crests, which are situated over mantle upwellings, and destroyed beneath the marginal trenches and island arcs which are the "sinks" in the system (Fig. 1). If such an upwelling were initiated beneath a continental area, the continent would be rifted and "drifted" apart and an intervening ocean basin formed as a result of lateral and symmetrical accretion of new ocean floor to the rift flanks; the rift ultimately becoming a mid-ocean ridge crest. The Atlantic and Indian Oceans might have been formed in this way during the past 200 million years, resulting in the separation of the surrounding continents, and implying average rates of lateral accretion or "spreading" of the ocean floor of 1-2 cm/yr. The East Pacific Rise might also be a

spreading ridge but initiated within a former oceanic area and therefore not constrained to be in a median position within the Pacific Ocean basin.

Magnetic Anomalies give Time Scale

In 1963, Matthews and I suggested² that sea floor spreading might be recorded in terms of the disturbances or "anomalies" in the Earth's magnetic field recorded over the ocean basins. If the Earth's magnetic field has reversed its polarity in the past then such reversals, coupled with spreading, would produce "avenues" of normally and reversely magnetized crust parallel to and symmetrically disposed about the mid-ocean ridge crests. The magnetization contrasts between these avenues would be capable of producing appreciable disturbances in the magnetic field at or above sea-level as measured by a ship or aircraft. By 1966 a geomagnetic reversal time scale had been deduced empirically for the past 3.5 million years from detailed palaeomagnetic and dating studies of subaerial lava flows and deep sea sediment cores. With the definition of this reversal time scale it was possible to demonstrate that the magnetic anomalies associated with ridge crests can be explained in terms of symmetrical spreading at essentially constant rates during the past 3.5 million years³. The distance from the ridge axis at which particular reversals are recorded makes it possible to deduce rates of spreading for all ridge crests for which magnetic data are available. Rates deduced in this way are indicated in Fig. 2 by the separation of the pair of solid lines adjacent to the dotted lines at the ridge crest. Integrated along the whole length of the mid-ocean ridge system these rates imply the formation of new oceanic crust at a rate of $2.6 \pm 0.2 \text{ km}^2/\text{year}$ (ref. 4).

The technicalities of both lava flow and sediment methods for determining the age of geomagnetic reversals are such that neither can extend the time scale back beyond 3.5 million years very readily or precisely. But the same sequence of magnetic anomalies away from ridge

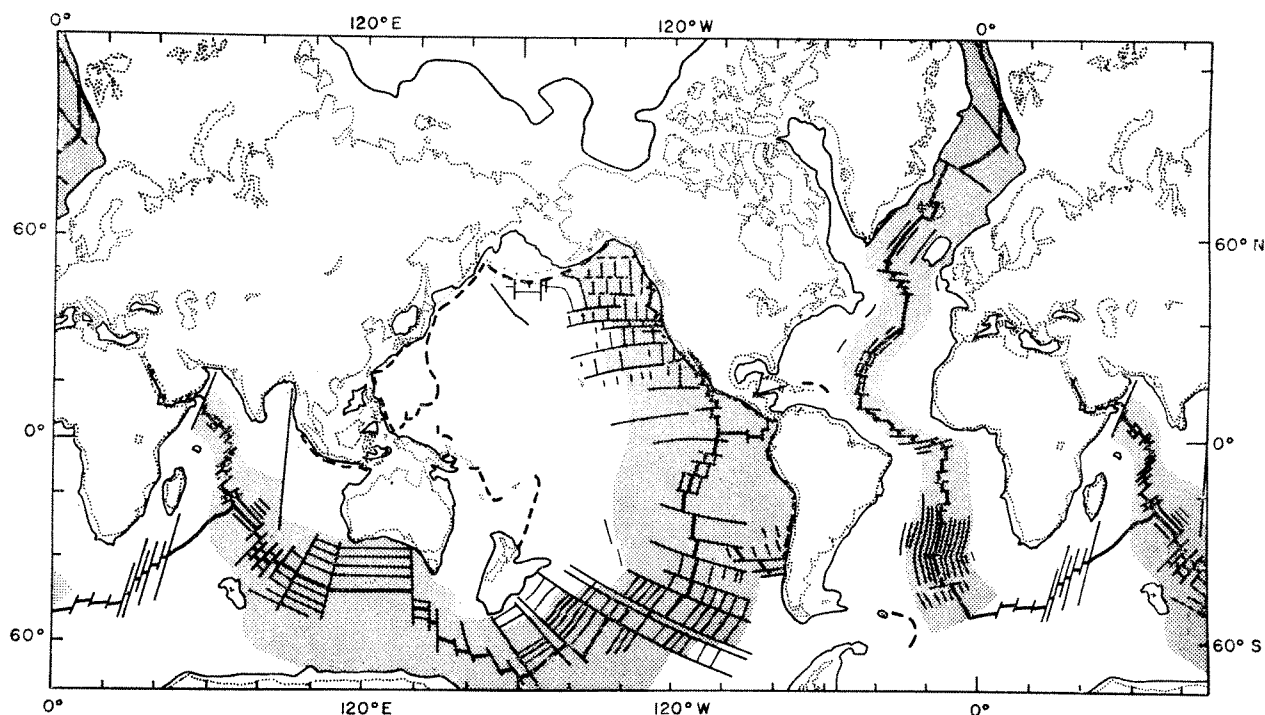


Fig. 1. Provisional attempt to delineate areas of continental and oceanic crust. Within the ocean basins, trenches are indicated by thick dashed lines, ridge crests by thick solid lines, and fractures (transverse to the ridge crests) and correlateable linear magnetic anomalies (parallel to the ridge crests) by thin solid lines⁵. Oceanic crust formed within Cainozoic time (that is the past 65 million years) is shaded.

crests is observed for thousands of kilometres across ridge flanks in all the major ocean basins implying that spreading and reversals of the Earth's magnetic field have occurred throughout an appreciable proportion of geological time. Areas in which this sequence of anomalies has been documented are summarized by families of parallel lines in Fig. 1 (ref. 5). To assign provisional ages to the older magnetic anomalies and thus to the underlying oceanic crust and to the implied reversals of the Earth's magnetic field, Heirtzler *et al.*⁶ assumed a constant rate of spreading of 2 cm/yr/ridge flank (that is, that deduced from the central anomalies) in the South Atlantic. In this way they were able to draw 10 million year "growth lines" for this and all other ridges across which the anomaly sequence has been recognized (Fig. 1).

Thus the confirmation of Hess's hypothesis in terms of the magnetic anomalies led to very specific predictions about the age and history of the ocean basins. With the advent of the US Deep-Sea Drilling Project two years ago it became possible to apply a very rigorous test of these speculations. In particular the primary objective of leg III of this so called JOIDES project was to test the spreading hypothesis and proposed geomagnetic reversal time scale. Eight sites were drilled across the Mid-Atlantic Ridge in the South Atlantic at approximately 30° S, and beneath the previously dated magnetic anomalies (Fig. 1). At all but one site the complete sedimentary column was penetrated and basalt "basement" recovered. Remarkably the palaeontological age of the oldest sediment recovered at each site, that is, immediately overlying or incorporated within the basalt, was found to be directly proportional to the distance of the site from the ridge axis⁷ (Fig. 3). Thus spreading about the South Atlantic ridge seems to have been continuous throughout the Cainozoic and at an essentially constant rate of 2 cm/yr/ridge flank, precisely the assumptions made by Heirtzler *et al.*⁶ in assigning ages to the magnetic anomalies.

This confirmation of the provisional ages assigned to the oceanic crust makes it possible to estimate the area of deep ocean floor formed by spreading during the Cainozoic, that is, the past 65 million years, as shown by the shaded area in Fig. 1. This necessitates considerable interpolation in the equatorial Pacific and Atlantic, where the magnetic anomalies are poorly developed because the Earth's magnetic field is essentially horizontal and parallels the trend of the ridge, but is probably quite an accurate estimate. It leads to the startling conclusion that perhaps 50 per cent of the present deep sea floor, that is, one-third of the surface area of the Earth, has been created during the most recent 1.5 per cent of geological time.

It seems extremely probable that all remaining oceanic areas are no greater than Mesozoic in age (that is, they were formed within the past 225 million years). The JOIDES drilling ship *Glomar Challenger* has occupied about 120 sites in the Atlantic and Pacific oceans and is currently in the Mediterranean; at no site so far has the age of the material recovered conflicted with the concept of sea floor spreading. In the western Pacific and western North Atlantic several sites were specifically chosen in the hope of sampling some of the oldest sediment in the ocean basins. The oldest sediment recovered, however, is middle Jurassic in age (that is, about 160 million years old). This was obtained recently in several holes just north of the Bahama Bank in the western North Atlantic⁸, and fits well with the previously suggested age of 180–190 million years for the initiation of drift between the east coast of the United States and north-west Africa^{9,10}.

It is now becoming apparent from palaeomagnetic studies on land that during the late Mesozoic or Cretaceous period (65 to 135 million years ago) there were very few reversals of the Earth's magnetic field¹¹. This is in great contrast to the Cainozoic, and would explain why areas of presumed Cretaceous ocean floor, for example, in the

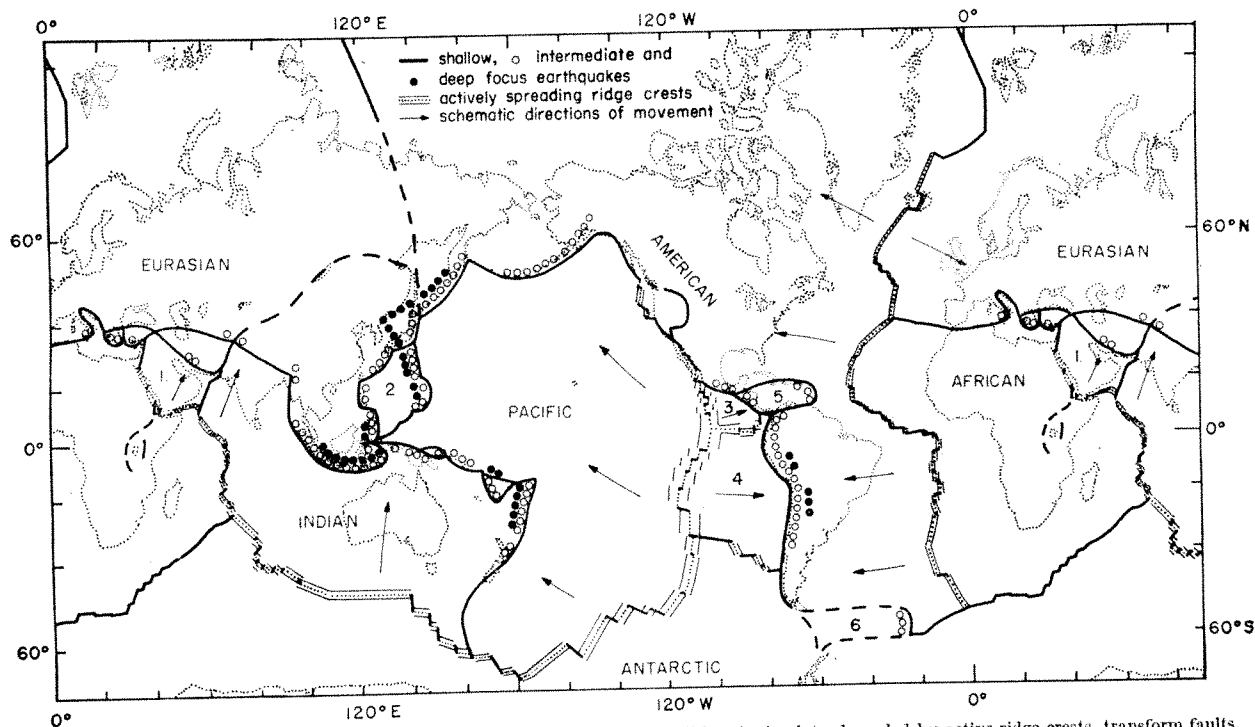


Fig. 2. Summary of the seismicity of the Earth²¹, and thus of the extent of lithospheric plates bounded by active ridge crests, transform faults, trench systems and zones of compression. The six major plates are named; the following minor ones are numbered: (1) Arabian; (2) Philippine; (3) Cocos; (4) Nazca; (5) Caribbean; (6) Scotia. Spreading rates at ridge crests are indicated schematically and vary from 1 cm/yr/ridge flank in the vicinity of Iceland to 9 cm/yr in the equatorial Pacific Ocean.

vicinity of Hawaii and along the margins of the South Atlantic, are "quiet" magnetically and devoid of correlatable linear anomalies. (The South Atlantic is thought to have opened up 110–120 million years ago^{9,10}.) To the southeast of Japan^{12,13} and in the North Atlantic, off the coast of the United States¹⁴ and northwest Africa¹⁵, linear magnetic anomalies have been mapped over even older ocean floor and are thought to reflect geomagnetic reversals during Jurassic, that is, mid-Mesozoic, time.

As I have already intimated, sea floor spreading has been superseded within the past few years by the even more potent and successful concept of plate tectonics. This has derived from a better understanding and more detailed consideration of the geometry of spreading and the seismicity of the Earth, the two phenomena being intimately related.

In 1965 J. Tuzo Wilson¹⁶ noted that within the framework of sea floor spreading a new class of faults or fractures in the Earth's crust is possible, their seismically active lengths terminating at ridge crests or trench systems (Fig. 2). This formulation of "transform faults" led to the realization by Morgan¹⁷, and McKenzie and Parker¹⁸ that the world-wide geometry of spreading and drift can be expressed, to a first approximation, in terms of relative movements between a limited number of rigid plates bounded by ridge crests, trench and mountain systems, and transform faults (Fig. 2). In a sense this is no different from the assumption made by Carey¹⁹ and Bullard *et al.*²⁰ in fitting together the Atlantic shelf edges of Africa and South America, but the plate tectonic hypothesis is more general. Thus the current seismicity of the Earth²¹ defines the current tectonic activity in the form of spreading and crustal tension at ridge crests, underthrusting and compression in the trench and mountain systems, and lateral translation without change in surface area along transform faults. Because the direction and rate of

spreading at ridge crests can be determined from the transform faults and magnetic anomalies respectively¹⁷, it is possible to make a global analysis of plate movements and predict the direction and rate of underthrusting in the trench and mountain systems²². Such directions are found to correlate well with the slip vectors deduced from focal mechanism solutions for some of the larger shallow earthquakes, and the rates with the maximum depths to which earthquakes occur beneath the trench systems²³. Presumably the latter reflect a thermal relaxation effect as

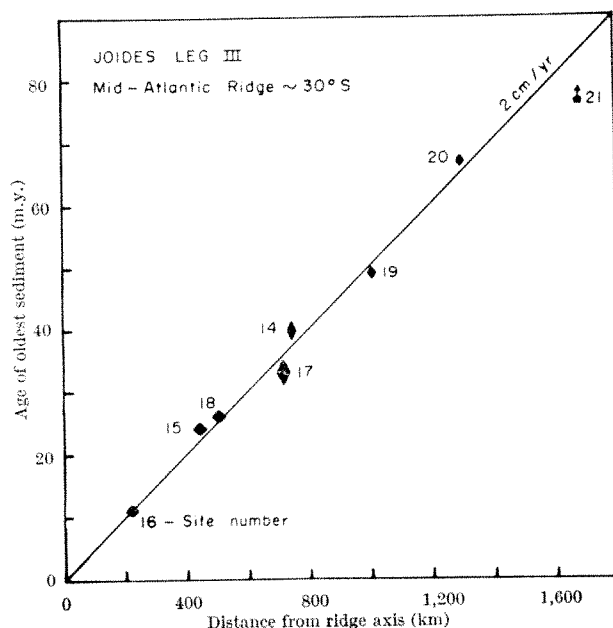


Fig. 3. The age of the oldest sediment recovered at each site plotted against the distance of the site from the ridge axis. With the exception of sites 17 and 18 all sites were drilled to the west of the ridge axis. The diamonds indicate the estimated error in the age and distance determinations. Basalt basement was not reached at site 21 (ref. 7).

cold oceanic crust and upper mantle is thrust down into hotter mantle and gradually assimilated.

It can now be seen that the earlier terms continental drift and sea floor spreading are inappropriate in that individual plates typically include both continental and oceanic crust, although a few are entirely oceanic (Fig. 2). Moreover, it has been realized that the Earth's outermost skin, or "lithosphere", which has appreciable strength and rigidity, extends well beneath both continental and oceanic crust to a depth of approximately 70 km²⁴. At ridge crests the lithospheric plates are thinned by the elevation of the geotherms as a result of mantle upwelling and emplacement; beneath the trench systems the oceanic plate plunges downward, typically at an angle of about 45° to the other plate, as indicated by the deep focus seismicity²⁵ (Fig. 2).

Plate Tectonics

The past year has seen important new developments and applications of the concept of plate tectonics. Its originators, McKenzie and Morgan, have collaborated²⁶ to formulate the geometry and evolution of triple junctions: points at which any combination of the three elements, ridge crests, transform faults or trenches come together. Of the various permutations perhaps the ridge-ridge-ridge type is the simplest. There is at least one example of such a triple junction within the present ocean basins, to the west of the Galapagos Islands (Fig. 2). Surprisingly, neither this nor other present day triple junctions had received much attention until recently. That to the west of the Galapagos Islands was investigated by scientists from Princeton University and the US Naval Oceanographic Office last May (reported in the *New York Times* on July 9, 1970) and seems to have a simple geometry, as predicted by plate tectonics, and a spectacular bathymetric expression. These findings lend added credence to the suggestion of Pitman and Hayes²⁷ and Hess and myself¹⁰ that the so called magnetic bight of the North Pacific, immediately south of the Aleutian Islands (Fig. 1), was formed at a triple-ridge junction approximately 65 million years ago.

Regional studies of seismicity, including focal mechanisms solutions, continue to verify the essentials of plate theory and to add details in complex areas. Recent areas studied include the East and West Indies^{28,29}, the Red Sea³⁰ and a particularly instructive analysis for the complex Mediterranean area by McKenzie³¹. The plate boundaries deduced by McKenzie for the eastern Mediterranean are incorporated in Fig. 2. With the definition of the geometry of transform faults and ridge crests along the Chile Rise^{32,33} only one major length of the mid-ocean ridge system remains inadequately defined. This is the south-west Indian Ocean ridge to the south-east of South Africa. Recently a South African expedition made a limited but detailed geophysical survey in the vicinity of Marion Island which may well provide this final piece of the jig-saw puzzle. Earlier data on the bathymetry, magnetism and sediment distribution across this ridge suggest that it is either dormant³, or was rejuvenated recently and is spreading very slowly. The latter is perhaps more probable, for the ridge crest is quite active seismically.

Island Arcs

An interesting and important new development within the context of sea floor spreading and plate tectonics is the suggestion that a process equivalent or analogous to

spreading at ridge crests may be occurring, probably on a more limited scale, behind island arcs. This concept has been documented in considerable detail for the Tonga-Kermadec arc by Karig³⁴ who suggested that it might also be applicable to the Marianas and other island arc systems with comparable morphologic provinces. Just more than a year ago, on JOIDES leg VI, two sites were drilled in the Philippine Sea on the inner arc behind, that is, west of the Marianas arc. These provided rapid confirmation of Karig's hypothesis in that they revealed a stratigraphy comparable with that beneath Guam on the arc proper, the intervening basin being flooded by a very thin veneer of sediments with presumably a very young volcanic basement beneath³⁵. It is not known whether such areas are characterized by linear magnetic anomalies, but it seems probable that the process of formation of this new oceanic crust is somewhat analogous to that occurring at ridge crests, although it may be taking place more slowly and over a broader and more diffuse zone.

In spite of the vast accumulation of near-surface evidence in favour of the hypotheses of sea floor spreading and plate tectonics we are little nearer to understanding the process or processes at depth by which they are initiated and maintained. Thermal convection in some form is probably the only mechanism capable of supplying the energy requirements, although convection in this context should be interpreted in its broadest terms, that is, as the generation of horizontal temperature gradients which result in gravitational instabilities and thus vertical transport of hot and cold material³⁶. Plate tectonics, however, make it clear that there can be no simple relationship between mantle convection currents and surface features, as Hess envisaged. The cold descending slab of oceanic lithosphere landward of the trench systems is clearly a source of gravitational energy and convection as I have defined. That such a "sinker" exists and is an important driving force is reflected in the tensile nature of intermediate focus earthquakes beneath certain trench systems³⁷.

Present knowledge of the physical properties of the Earth's mantle is far from perfect, particularly with regard to temperature and viscosity, two all-important parameters; but it seems probable that convection is only possible in that part of the upper mantle which is nearest to its melting point. This region, sometimes termed the "asthenosphere", is thought to be between depths of approximately 70 and 400 km, the latter depth being particularly uncertain. Convection in this shell would produce two further effects: a net viscous drag on the lithospheric plates above and, as a result of thermal upwelling at ridge crests and the resultant elevation of ridges, the flanking plates are situated in gravity potential gradients which tend to pull them downhill³⁸.

There is therefore no lack of possible driving mechanisms: plates may be pushed apart by mantle upwelling, pulled apart by sinkers beneath the trench system, carried by convection currents in the asthenosphere or slide under the influence of gravity. Moreover, stresses set up between and within plates may in part determine their motions; this may be particularly relevant in the case of small plates. It seems probable that plate movements are not solely attributable to any one of these effects but result from a combination of all of them acting in varying degrees on individual plates.

More than fifty years ago Taylor and Wegener formulated the concept of continental drift within the more respect-

able and plausible geological framework of uniformitarianism, rather than catastrophism. Most geologists, however, rejected the hypothesis on the grounds that the geological evidence was equivocal and because geophysicists maintained that there was no plausible mechanism to account for drift. Surprisingly, with the advent of compelling evidence for sea floor spreading in the mid-1960s, geophysicists, both theoretical and applied, were the first to accept the new findings and review their implications for their own areas of interest. Thus the wave of activity and re-interpretation which had been initiated in the area of marine geology and geophysics spread out and through seismology and all areas of theoretical and applied geophysics. Only within the past year or two has this excitement reached the classical areas of geology relating to structure, magmatism, sedimentation and metamorphism. Symptomatic of this was the seance held under the auspices of the Geological Society of America at Asilomar, California, during December last. Here a limited number of invited geologists met to discuss "the meaning of the new global tectonics for magmatism, sedimentation and metamorphism in orogenic belts"³⁹. Illustrative of the concepts discussed at this conference are the re-interpretations of Dewey⁴⁰ and Moores⁴¹ of the Appalachian/Caledonian and western Cordillera Orogens respectively. Many of the basic tenets of these new syntheses were advanced many years ago by, for example, Takeuchi and Uyeda⁴² in recognizing the possibility of present day regional metamorphism in the Japanese Island Arc; by Hess⁴³ in postulating that ultramafic bodies in mountain belts were emplaced in the initial stages of orogeny, and, perhaps most significantly, by Hess again in stating in 1940 (ref. 44) that "perhaps no greater misconception exists in geology than that geosynclines localize mountain building".

Lunar Samples

The study of lunar samples returned to Earth by Apollo missions 11 and 12 has enabled us not only to gain insight into the history and composition of the Moon, but also to see the history of the Earth and some of its geochemical peculiarities in a new and refreshing light. Of the initial results⁴⁵ the great age of the lunar maria came as a surprise to many, as did the occurrence of anorthosite fragments in the lunar "soil". The discovery that anorthosite (an essentially mono-mineralic calcium-aluminium-silicate rock) is an important rock type on the Moon and may well constitute the lunar highlands⁴⁶ illustrates the way in which the study of lunar materials has heightened interest and added a new dimension to the study of similar materials on the Earth. Anorthosites occur terrestrially but present something of a geological enigma in that their distribution is limited in space and time. On a pre-drift reconstruction of the northern and southern continents anorthosite outcrops plot in two belts, one striking south-west-north-east across North America and extending into Scandinavia, and the other crossing Africa, Madagascar and India. All these anorthosites yield radiogenic ages within the range 1,100 to 1,700 million years⁴⁷. Terrestrial anorthosites are thought to form in conditions of high pressure and temperature, probably at the base of tectonically thickened continental crust⁴⁸; to add to the enigma they occur in orogenic belts, such as the Grenville of North America, which are devoid of Hess's "Alpine-type" ultramafics. The full significance of the occurrence of anorthosite on the Earth and Moon is still not understood and poses an intriguing problem for

the future. The recent publication of a collection of research papers on the terrestrial anorthosite problem is timely, if fortuitous⁴⁸.

After a year of intensive study of the lunar rock samples Schreiber and Anderson⁴⁹ provided a little light relief in noting that the velocity of sound in lunar rocks is comparable with that in terrestrial cheeses. Cheeses, however, obey the velocity-density relations proposed for Earth rocks, whereas lunar rocks deviate widely from them because of their high porosities.

For many years the Earth sciences were the poor sister of the physical sciences. Ironically, at a time when most other fields in this area are suffering a "recession" and having difficulty in placing their newly graduated PhDs, the geosciences are enjoying a revolution in thinking within their traditional areas of study and facing new frontiers as a result of the space programme and heightened activity in the study of the ocean basins and the physical environment as a whole.

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Highlights of High Energy Physics

by

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Data provided by accelerators in various parts of the world have given high energy physicists plenty to think about during the past year.

In late 1967 the 70 GeV proton synchrotron at Serpukhov in the USSR began operation, just in time for the fiftieth anniversary of the October revolution. This is presently the accelerator of the highest energy in the world, and during the past year the first experiments have been reported from Serpukhov¹⁻⁵. They have been performed by a joint group from CERN and the USSR, using a negative particle beam, bending outward from an internal target; a positive particle beam must await the extraction of the proton beam; high energy positive particles at 0° from an internal target will bend inward further into the machine.

The first experiments have been measurements of total cross sections for three negative particles π^- , κ^- , \bar{P} ; on hydrogen, deuterium, and nine elements up to uranium, at energies of 20–65 GeV. The excitement in these cross sections arises from Pomeranchuk's conjectures⁶, often dignified by the name theorem, that the total cross sections of any particle on a nucleon become independent of energy at high energies, and that the cross section of a particle becomes equal to that of its antiparticle.

Earlier experiments at Brookhaven⁷ and CERN had shown a steadily decreasing cross section in most cases, as shown for π mesons in Fig. 1. The cross sections for π^+P and π^-P collisions were still 5 per cent apart at 20 GeV, and it is not certain whether they were extrapolating to the same value; a simple plot $\sigma = A + B/E^{1/2}$ (Fig. 2) showed a slight difference, but more complex extrapolations could be made where the difference in cross sections vanished at infinite energy as Pomeranchuk suggested. One of these, by Barger⁸, is shown as a solid line in Fig. 1.

Surprising Cross Sections

The new data apply to negative particles only; however, the π^+P cross section should equal the π^-N cross section because these states have the same isotopic spin and the charge independence of the nuclear forces makes the cross sections equal. The π^-N cross section is obtained from the π^-D cross section by subtracting the π^-P cross section and making a small correction for the shadowing of one nucleon by the other. These data are also plotted in Figs. 1 and 2. It is obvious that the earlier expectations are much changed; the cross sections are flattening off much faster than any simple formula can describe; and the two cross sections are not approaching each other. The result of the excessively

rapid flattening of the cross section also holds for $\bar{P}P$, $\bar{P}N$, κ^-P and κ^-N cross sections, though here it is not possible to compare particle and antiparticle cross sections until a positive particle beam is available; the charge independence argument possible for π^-N and π^+P cross sections does not work for these combinations.

These new data have led to a flurry of theoretical activity. While it was believed that cross sections obeyed the Pomeranchuk "theorem" at high enough energies, the interest in the total cross sections at high energies

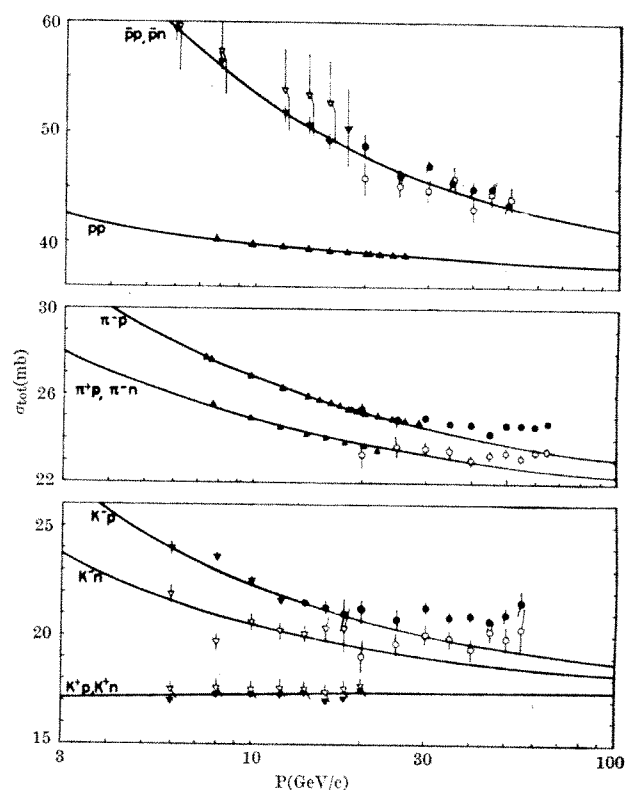


Fig. 1. Total cross sections for particles on protons and neutrons. Solid lines are fits* to old data. The fits extrapolate, as $E \rightarrow \infty$, 4 mb (PP), 33 mb ($\pi^\pm N$), and 25 mb ($K^\pm P$).

Target
p n
▲ ▼ Foley (AGS)
▼ ▽ Galbraith
● ○ IHEP-CERN
— predictions made by Barger

waned; it seemed that this branch of high energy physics was complete. Now there are several new ideas. One of them⁹, based on a complex application of field theory, suggests that the cross sections increase with energy logarithmically; but that particle and antiparticle cross sections have, at high energies, a constant difference. This increase of cross section should, in this model, be accompanied by many low energy π mesons, produced at large impact parameter. The word "pionization" has been used to describe this process. This theory therefore makes a prediction (a phenomenon all too rare in high energy physics) that these low energy pions will be found in high energy collisions, and in increasing number as the energy increases.

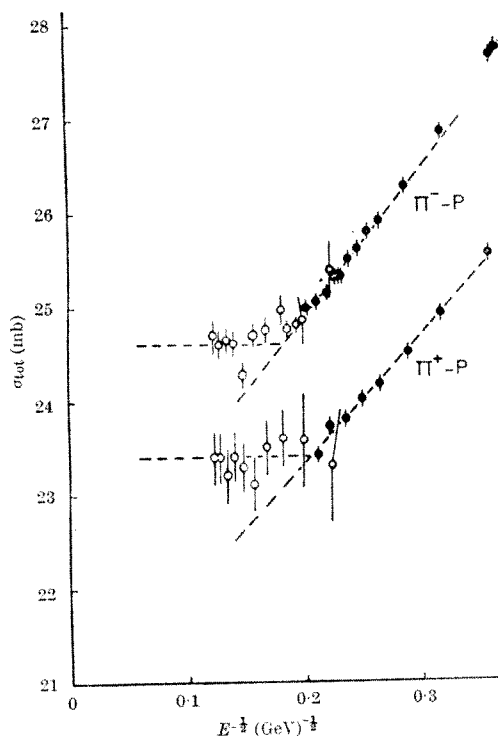


Fig. 2. π^-P and π^+P ($=\pi^+N$) total cross sections plotted against $E^{-1/2}$ to display clearly the discontinuity. \bullet , Foley *et al.* (AGS); \circ , Allaby *et al.* (IHEP).

Results are eagerly awaited from the positive particle beam at Serpukhov which should be available in the next two years; this should confirm the $\pi^\pm P$ behaviour for π^\pm and P^\pm . Also in two years, the 30 GeV intersecting storage rings (ISR) at CERN, and the 200–500 GeV accelerator at the National Accelerator Laboratory, Batavia, should be working. It may be possible to see the cross sections turn upwards; even more important, pionization, if it occurs, should be directly measurable at these energies.

Electron-Positron Interactions

The operation of the electron-positron storage ring (ADONE) at Frascati was described last year in *Nature*¹⁰. The experimental programme was set back by a four month strike, but preliminary results were presented to the recent international conference on high energy physics at Kiev (from groups led by M. Conversi, G. Salvini, V. Silvestrini and A. Zichini).

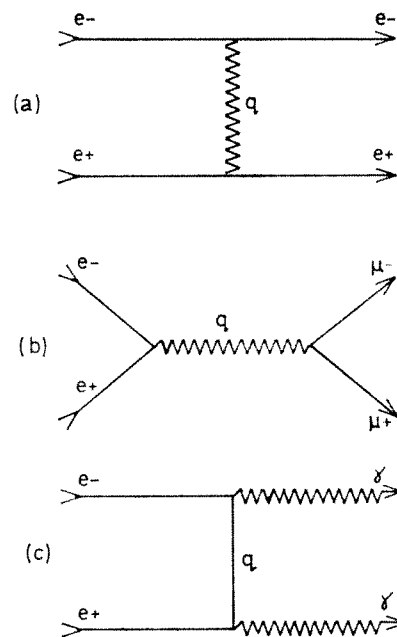


Fig. 3. Typical Feynmann diagrams showing the sensitivity to different propagators. (a) Space-like photon propagator $q^2 = 4E^2 \sin^2\theta/2$; (b) time-like photon propagator $q^2 = -4E^2$; (c) space-like electron propagator $q^2 = 4E^2 \sin^2\theta$.

Technical difficulties restricted the first operation to energies (E) of 800 to 1,000 MeV in each beam, making a total energy ($2E$) of 1,600 MeV to 2,000 MeV in the centre of mass. It was originally intended to keep the electron and positron beams apart by an electrostatic field and to make them meet and cross at an angle in each interaction region; the length of the interaction region would be limited thereby. For reasons not yet fully understood, this led to instabilities. Accordingly the beams are not kept apart, but interact whenever one radio frequency "bunch" meets another—at six places of 30 cm length around the machine. Around four of these interaction regions experimental equipment is placed, and four experiments proceed simultaneously for sixteen hours a day. The most exciting result to come from these measurements is a large cross section for producing hadrons—presumed to be mostly π mesons. The electron and positron annihilate; the subsequent interaction with hadrons is described by a virtual photon which is time-like. We can produce all known charged particles in pairs, $\mu^+\mu^-$, e^+e^- , $\pi^+\pi^-$ and so on. This is done without any intermediate hadron state. It is a very clean way of studying specific hadron properties, similar to the study of proton sizes by electron scattering.

Spark chambers are set up on either side of the interaction region, and in a typical case they are triggered whenever two charged particles are observed on opposite sides, which pass through two counters each. There are many more events due to cosmic ray events than due to the beam-beam interactions, but when the tracks are examined in detail, we pick those that come from the interaction region, and those which are in time with the interaction (which can be measured from the phase of the radio frequency). These restrictions reduce the cosmic ray background to manageable proportions. The μ mesons are identified by the fact that they penetrate a lot of absorber; electrons by the fact that they produce showers in lead absorber, and hadrons by the

fact that they produce a single track with no showers, and do not penetrate as much absorber as muons.

Pairs of particles will always be produced collinear, for the centre of mass system and the laboratory system coincide. More than 5,000 electron-positron pairs and μ pairs have been so identified. About fifteen hadron pairs are also seen, but some of these can be confused with electron pairs which fail to shower. So far we can say that the π meson form factor lies between the point form factor and the rho dominance form factor.

But when three or more hadrons are produced they are easily recognized because the tracks are not collinear. Sometimes two non-collinear tracks are observed; sometimes three and four, and in one case, five tracks. Since the solid angle subtended by the detector systems is small, $1/4$ of 4π , this implies a large average multiplicity ($7-1/2$) of the interaction. This already is surprising; with an average centre of mass energy of 1,800 MeV this leaves only 240 MeV per particle; only 100 MeV more than the rest mass of a pion!

The total cross section can be estimated; it turns out to be five times greater than that for pion pairs with point pions. Within the limited range it seems independent of energy.

Often general relationships relate one cross section with another; Regge pole theory relates high energy cross sections with low energy particle states of different angular momentum; current algebra has related the low energy production of pions with a sum over high energy cross sections. No such relationship exists here, however. Early in the development of current algebra it was thought that the commutator between two electromagnetic currents $[j_\mu(x), j_\nu(0)] = 0$. Schwinger showed that this commutator is indeterminate and, further, that this commutator equals the cross section for an electron and a positron to produce a hadron. These terms, promptly dubbed "Schwinger terms", cannot therefore be predicted from previous experiments.

Various models can be made to aid anyone who wishes to speculate; the particles might all behave like pions; because the number of possible particle pairs increases with energy, the total cross section would then become large. Or they could have form factors with the cross sections reduced by the square of the form factors $[F(q^2)]^2 = [F(4E^2)]$. The usual guesses were a "vector dominance" form factor

$$F(q^2) = \frac{m_e^2}{m_e^2 + q^2} [q^2 = -4E^2]$$

with $m_e^2 \simeq 0.55$ (GeV) and where it was assumed that the hadron interacts only through the intermediary of a rho meson. This might work for the pion (and indeed it fits in detail the production of rho mesons). For the proton, however, this form factor should also fit electron-proton scattering experiments where, by contrast, it is found experimentally that

$$F(q^2) \simeq \left[\frac{0.71}{0.71 + q^2} \right]^2$$

At $q^2 = -4$ GeV² these are $1/7$ and $1/20$ respectively and the cross sections $1/49$ and $1/400$. For the total cross sections, into all possible hadronic states, various speculations have been made that this total cross section equals that for a point pion; increases as E^2 , or decreases as E^2 . Experiment is now beginning to settle these questions.

These results show that there is a wealth of new information to be gained from high energy colliding beams. New devices at Novosibirsk, CEA, DESY and SLAC are expected to join in this investigation within the next few years.

Quantum Electrodynamics

Quantum electrodynamics (QED) is quantitatively the most successful physical theory. For example, experiments on the Lamb shift now agree with theory to within the experimental error of 1.0 MHz; this can be compared with the $^2p_{1/2} - ^2s_{1/2}$ transition frequency of 1,000 MHz, the $^2p_{3/2} - ^2s_{1/2}$ transition of 9,000 MHz or the $^2p - ^1s$ transition of 10^9 MHz. The precision is fantastic.

The theory has within it, however, the seeds of its own destruction; it seems to be fundamentally divergent at small distances (large momentum transfers). This is the domain of the high energy physicist and there is an incessant search for such deviations. The procedure is to measure some process which can be calculated by QED. Any deviation is expressed as a modification of an appropriate propagator in the Feynmann diagram

$$1/q^2 \rightarrow 1/q^2 + \frac{1}{q^2 + \Lambda^2}$$

Such a modification can be due to a heavy photon of mass Λ . Lee and Wick¹¹ particularly emphasize the importance of this.

The new colliding beam measurements at Frascati give the latest and cleanest tests of the theory; the production of electron-positron pairs, μ pairs and γ pairs are sensitive respectively to modifications of the space-like photon propagator, a time-like photon propagator, and a space-like electron propagator. In each case they find $\Lambda > 3$ GeV. The only comparable measurement is the two year old measurement of the anomalous moment of the muon ($g-2$) at CERN where the finding for a time-like photon propagator is $\Lambda > 5$ GeV.

At one time it was hoped that strong interactions could give a deviation giving Λ somewhere close to the nucleon mass; no one knows where to look for such a deviation now; the divergences do not become serious until we reach energies of e^{137} electron masses which is much larger than presently conceived accelerators!

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Rous Sarcoma Virus: a Function required for the Maintenance of the Transformed State

by

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A temperature sensitive RNA tumour virus mutant has been isolated whose properties indicate that part of the viral genome is required for transformation but not for growth.

It now seems likely that the genome of DNA or RNA-containing tumour viruses persists in cells transformed by them, at least in most cases and perhaps in all. In the case of DNA tumour viruses the presence of viral DNA can be demonstrated by DNA-RNA hybridization or by the production of infectious virus following fusion with cells which allow virus growth (for reviews, see refs. 1 and 2). Cells transformed by RNA tumour viruses may release infectious virus while continuing to divide³⁻⁶, for these viruses, unlike the DNA tumour viruses, are not cytotoxic. In certain situations, depending mainly on the type of cell and virus strain used, non-infectious particles are released which can be detected by electron microscopy or by labelling with radioactive precursors^{7,8}. Even in those cases where no particles are detectable, the genome can frequently be rescued by co-cultivation or fusion with permissive cells⁸⁻¹⁰.

The role of viral genes in producing the characteristic physiological changes of transformation is, however, still unclear. In the case of the DNA tumour viruses it is not yet known whether the continued expression of viral genes is required to maintain the transformed state^{1,2}. A considerable number of temperature-sensitive mutants of polyoma have been isolated¹¹⁻¹³, but none of the mutants so far described is defective in the ability to stimulate DNA synthesis in confluent cells, to produce abortive transformation, or to maintain the transformed state¹¹⁻¹⁵. In the case of the RNA tumour viruses, on the other hand, a specific role for viral genes in transformation is suggested by comparison of the leukosis and sarcoma viruses. These viruses, which are structurally and chemically indistinguishable¹⁶, grow in fibroblast cultures but only the sarcoma viruses transform them. Goldé¹⁷ and K. Toyoshima, R. R. Fris and P. K. Vogt (manuscript in preparation) have recently shown that irradiation of certain strains of avian sarcoma virus results in the formation of viruses which retain the ability to grow but are no longer able to transform. These experiments indicate that part of the genome of RNA tumour viruses is required for transformation but not for growth. I now describe the isolation of a temperature-sensitive mutant of the Schmidt-Ruppin strain of Rous sarcoma virus the properties of which indicate that the mutation affects a function required for the maintenance of the transformed state but not for the growth of the virus.

Isolation of the Mutant

The methods used for the growth and assay of virus stocks were those previously described¹⁸, except that the agar overlay used in the assay was supplemented with 3 mg/ml. glucose and an extra 4 ml. of agar overlay was added after 4 days incubation. C/O or C/B (ref. 19) chick fibroblasts were used throughout. The method used to clone virus stocks involved picking clones of transformed cells growing in agar suspension: transformed cells grow into large colonies in agar suspension whereas normal cells either do not divide or form only small colonies^{20,21}. Cells were plated at 1.5×10^6 per 10 cm plastic dish (Falcon Plastics) in medium 199 (Grand Island Biological Co.) containing 2 per cent tryptose phosphate broth

(TPB, Difco) and 1 per cent calf serum (Microbiological Associates). The next day the medium was removed and the cells were infected with 0.5 ml. of a virus stock diluted to between 2 and 20 focus forming units (f.f.u.) per ml. After 1 h adsorption the cells were trypsinized and re-suspended at 1.5×10^6 per ml. in medium 199 or Scherer's medium containing 10 per cent TPB, 4 per cent calf serum, and 1 per cent chicken serum (Microbiological Associates; heat inactivated for 60-90 min at 60° C). 0.5 ml. of this cell suspension was then added to 1 ml. of the same medium containing 0.6 per cent agar and poured over 5 ml. base layers of the same agar mix. (The transformed clones tend to grow faster in medium 199 than in Scherer's but are less readily distinguished from the small clones of normal cells which also grow to a greater extent in medium 199.) After incubation for about a week at 38°-39° C or 2 weeks at 36° C clones were picked onto growing monolayers of cells, using 2 μ l. capillaries (Drummond Microcaps) or drawn-out Pasteur pipettes. The cells were then grown and transferred when necessary until extensive transformation was obtained.

A stock of Schmidt-Ruppin virus, SR-RSV-A, free of avian leukosis viruses, was kindly provided by Dr W. Levinson, who had previously obtained the virus from Dr P. K. Vogt. The virus was recloned by the method described above. The recloned Schmidt-Ruppin virus did not plate on RAV1-infected or C/A cells, and chicken antisera prepared against it neutralized RSV(RAV1) but not RSV(RAV2), confirming that this virus belongs to the A subgroup^{19,22,23} of avian tumour viruses. It has been reported²⁴⁻²⁷ that the Schmidt-Ruppin strain, unlike the Bryan strain, is not "defective", in the sense that no helper virus is required for the production of virus infectious for the commonly used C/O or C/B type fibroblasts. Stocks of the Schmidt-Ruppin strain can be prepared in which leukosis viruses are not detectable by endpoint dilution procedures²⁴⁻²⁷; infection at low multiplicities results in the formation of foci^{24,25,27} or pocks on the chorio-allantoic membrane²⁶ which all release infectious virus. To confirm that cells singly infected by SR-RSV-A release infectious progeny, cells were infected at a multiplicity of about 10^{-5} with SR-RSV-A and also with the helper-dependent Bryan strain, RSV(RAV1). The infected cells were plated in agar suspension and clones picked as described. As expected, all SR-RSV-A infected clones released infectious virus (6 out of 6 in one experiment, 20/20 in another) whereas most of the Bryan clones (5/6) did not.

To isolate mutants of the virus, the mutagen N-methyl-N'-nitro-N-nitrosoguanidine ("nitrosoguanidine", MNNG) was used: this mutagen has been shown by Hanafusa²⁸ to lead to the production of mutants of Bryan RSV β (0) which are unable to yield progeny infectious for quail cells. A crude stock of SR-RSV-A, titre about 10^6 f.f.u./ml., was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.0) containing 0.002 M EDTA and 2 mg/ml. MNNG (Aldrich Chemical Co., dissolved at 100 mg/ml. in dimethylsulphoxide immediately before use). After 10 min incubation at 30° C the mixture was chilled and dialysed extensively against standard buffer (0.1 M

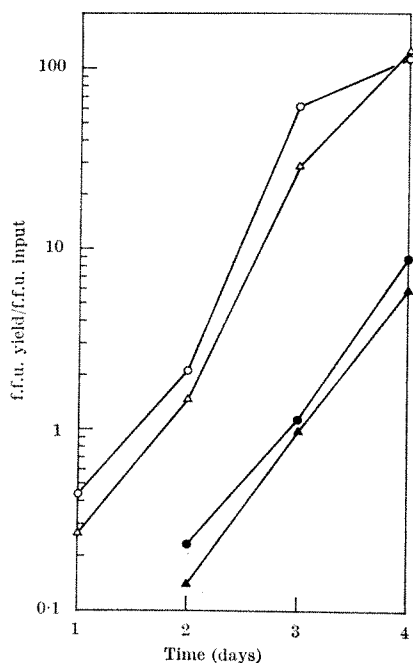


Fig. 1. Growth of SR-RSV-A and T1 at 41° C and at 36° C. Cells were seeded in four bottles (5×10^5 cells per bottle in medium 199 containing 2 per cent TPB and 1 per cent calf serum) and infected after 24 h with $2-4 \times 10^4$ f.f.u. of either SR-RSV-A or T1. After the virus had adsorbed for 1 h, the cells were washed and the medium was changed to medium 199 plus 10 per cent TPB and 5 per cent calf serum. One bottle of each pair was held at 36° C, the other at 41° C, and the medium was changed every 24 h. After 4 days the cultures kept at 36° C were partially transformed, the SR-RSV-A-infected culture at 41° C was completely transformed, whereas the T1-infected cells at 41° C showed no transformation. The virus in the medium collected each day was titrated at 36° C. The virus yield is expressed as the amount of virus in 5 ml. medium divided by the amount of virus used to infect the cells (2.1×10^4 f.f.u. of SR-RSV-A; 3.4×10^4 f.f.u. of T1). △, SR-RSV-A at 41° C; ▲, SR-RSV-A at 36° C; ○, T1 at 41° C; ●, T1 at 36° C.

NaCl-0.01 M Tris-HCl (pH 7.6)-0.001 M EDTA). The titre of the mutagenized stock was approximately 5×10^2 f.f.u./ml., representing a survival of about 10^{-3} compared with a control without nitrosoguanidine. The surviving viruses were cloned at 35°-36° C using the agar suspension method described. Two hundred and sixty clones were picked and the resulting virus stocks, grown at 35°-36° C, were tested for their ability to produce foci at 36° C and 41° C. Six were found to be unable to produce foci at 41° C; one of these mutants, T1, was re-cloned, and the properties of this mutant will be described. Because the permissive and non-permissive temperatures are only 5° C apart the characterization of this mutant was carried out using cultures grown in sealed 25 cm³ plastic bottles (Falcon Plastics) immersed in water baths controlled to an accuracy of $\pm 0.05^\circ$ C by mercury contact thermoregulators.

Growth of the Mutant at the Non-permissive Temperature

The ability of the mutant to grow at the non-permissive temperature was tested as recorded in Fig. 1. There is no significant difference between the wild type and the mutant in the rate of accumulation of virus either at 41° C or at 36° C. Because Rous sarcoma virus is thermally inactivated at a significant rate, the amount of infectious virus in the medium depends not only on the rate of production but also on the rate of inactivation³. The rates of inactivation at 41° C of the wild type and of the mutant (grown at 41° C) were therefore measured. The survival of the wild type after 2 h was 46 per cent and after 4 h, 22 per cent; that of the mutant after 2 h was 44 per cent and after 4 h, 19 per cent. At 41° C there is no significant difference between the mutant and the wild type either in the rate of accumulation of virus or in the rate of inactivation, so that it may be concluded

Table 1. SUPERINFECTION OF T1-INFECTED CELLS GROWN AT 41° C

Superinfecting virus	No. of infected cells (per 100 mm plate) capable of forming foci at 41° C	
	Uninfected cells	T1-infected cells
None	0	4.0×10^2
SR-RSV-A	1.4×10^5	4.2×10^2
RSV(RAV1)	1.5×10^5	6.5×10^2
PR-RSV-C	1.8×10^4	2.8×10^4

Cells were infected with T1 at a multiplicity of infection of about 10^{-2} f.f.u./cell and these and a parallel culture of uninfected cells were grown at 41° C for 7 days with one transfer. Infected and uninfected cells were then plated at 1.5×10^5 /100 mm plate and after a day at 41° C a second infection was carried out with $2-10 \times 10^4$ f.f.u. of either SR-RSV-A, RSV(RAV1), or PR-RSV-C (Prague virus, a C group sarcoma virus kindly provided by Dr P. K. Vogt). After the virus had adsorbed for 1 h, the cells were washed with Tris-saline and then exposed to 0.05 M glycine-HCl buffer (pH 2.2) for 60 s, to inactivate adsorbed but unpenetrated virus²². After restoring the pH to neutrality with three washes of Tris-saline, the cells were incubated for 2 h in medium. The cells were then trypsinized and plated at various dilutions on sensitive cells to determine the number of infected cells capable of forming foci at 41° C; anti-SR-RSV-A antibody was added to the medium of the assay plates to reduce the background arising from the spread (before the agar overlay) of temperature-resistant revertants of T1.

that the mutation does not affect the growth of the virus. The mutant grown at 41° C was not able to form foci on subsequent plating at 41° C, indicating that the defect is not altered by growth at the non-permissive temperature.

At 41° C the behaviour of this mutant is similar to that of the avian leukosis viruses, which grow in fibroblast cultures without transforming them. Cells infected by an avian leukosis virus are in general resistant to superinfection by viruses of the same subgroup²¹, probably because of the blocking of receptors required for viral penetration. To determine if cells infected by the mutant also exhibit this subgroup-specific interference the experiments documented in Table 1 were performed. As expected, the T1-infected cells are resistant to superinfection by the A group viruses, RSV(RAV1) and SR-RSV-A, but not to infection by PR-RSV-C. The high degree of resistance to A group viruses indicates that almost all the cells are infected.

Effect of Mutation on Ability to Transform

The mutant grows at 41° C without producing any visible effect on the morphology of the infected cells. If the function which is defective at 41° C is required only to initiate the transformed state, it would be expected that T1-transformed cells growing at the permissive temperature would remain transformed when shifted to the non-permissive temperature. It was found, however, that T1-transformed cells when shifted to 41° C rapidly lost their rounded refractile appearance and assumed a normal or near-normal appearance, the change being largely complete within 4 h; cells infected by the wild type were not affected by the shift. This indicates that the mutant function is required to maintain the morphological alterations which are characteristic of the transformed state. The reverse change, from the normal to the transformed morphology, occurs when a T1-infected culture grown at 41° C until completely infected (as judged by the resistance of the cell population to superinfection) is shifted down from 41° C to 36° C. This change is slow, however, taking about 2 days; presumably this is at least in part because cellular metabolism is slow at the permissive temperature.

The effect of the mutation on the ability of the infected cells to grow in agar suspension was also investigated (Table 2). It can be seen that T1-infected cells do not form transformed clones at 41° C. Moreover, even if grown at 37° C for 4 days, they are unable to grow into recognizable transformed clones when shifted to 41° C. T1-infected cells maintained at 41° C for 4 days can, however, produce transformed clones when shifted down to 37° C. In a similar experiment, infected cells in agar suspension were shifted to 41° C after 9 days' growth at 37° C, by which time the transformed clones were clearly distinguishable. After incubation for a further 6 days, it was clear by inspection that clones produced by T1 after incubation for 15 days at 37° C were larger

Table 2. EFFECT OF TEMPERATURE ON THE GROWTH IN AGAR SUSPENSION OF CELLS INFECTED WITH SR-RSV-A OR T1

Temperature of incubation	No. of transformed clones per bottle	
	SR-RSV-A	T1
41° C for 16 days	310, 330	0, 0
37° C for 16 days	210, 222	209, 204
37° C for 4 days; then 41° C for 12 days	274, 270	0, 0
41° C for 4 days; then 37° C for 12 days	233, 220	197, 174

A preliminary experiment indicated that the optimum permissive temperature for the growth of T1-infected cells in agar suspension was 37° C, and that at this temperature the transformed clones become distinguishable from the small clones of normal cells after incubation for 5–6 days. Cells were therefore infected with SR-RSV-A or T1 (about 1,000 f.f.u. per 1.5×10^6 cells), suspended in Scherer's agar as described, and kept at either 37° C or 41° C. After 4 days, just before the transformed clones had become distinguishable some bottles were shifted from one temperature to the other. The number of transformed clones was counted after 16 days.

than those shifted to 41° C after 9 days. These results indicate that the mutant function is required to maintain growth in agar suspension.

Genes for Growth and Transformation

The properties of this mutant indicate that part of the viral genome is required for transformation but not for growth, because only transformation is affected at the non-permissive temperature. This is in agreement with the conclusions of Goldé¹⁷ and of Toyoshima *et al.*, who have shown that irradiation of avian sarcoma viruses by γ -rays or ultraviolet light leads to the production of non-transforming mutants which are able to grow as well as (or better than¹⁷) the wild type. Goldé (ref. 17 and personal communication) has proposed that the part of the genome which codes for the oncogenic functions includes information for the synthesis of an inhibitor of viral multiplication. The properties of T1 neither support nor contradict this proposal, but if such an inhibitor exists, the mutation in T1 cannot affect it, for the mutant grows at the same rate as the wild type at both permissive and non-permissive temperatures (Fig. 1).

Macpherson³⁰ has observed that BHK21 cells transformed by the Schmidt-Ruppin strain (presumably SR-RSV-D, see ref. 31) can give rise to non-transformed cells from which an active transforming genome cannot be rescued by injection into chickens. He suggested that this reversion arose from the loss or inactivation of the viral genome. It would follow that the presence or expression of viral genes is required to maintain the transformed state. This conclusion, in particular the idea that the expression of viral genes is required to maintain transformation, is supported by the properties of temperature sensitive mutants of avian sarcoma viruses which are unable to sustain transformation when the infected cells are transferred to the high temperature.

These mutants include the mutant described here and the mutants of B77 recently isolated by Toyoshima and Vogt³², which differ from T1 in that they are unable either to grow or to transform at the non-permissive temperature.

Further characterization of these and other mutants may help in identifying the biochemical changes in infected cells which are involved in transformation.

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Presence of DNA in Rous Sarcoma Virus

by

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DNA has been isolated from the virion of Rous sarcoma virus. It consists of two components which seem to correspond to the two species of DNA synthesized *in vitro* by the recently identified virion RNA-dependent DNA polymerase.

It is widely believed that cells transformed with Rous sarcoma virus (RSV) contain a DNA transcript of the viral RNA, the so called "provirus"¹. In spite of ample evidence that the bulk of the nucleic acid is RNA^{2,3}, it therefore seemed worthwhile to investigate the possibility that some DNA is enclosed within the virion, especially

now that RSV has been shown to contain an RNA-dependent DNA polymerase (refs. 4, 5 and J. M. Bishop *et al.*, manuscript in preparation).

Purified RSV (Schmidt-Ruppin strain) was doubly labelled with ³H-thymidine and ¹⁴C-uridine and analysed on sucrose gradients using both isopycnic (Fig. 1A) and

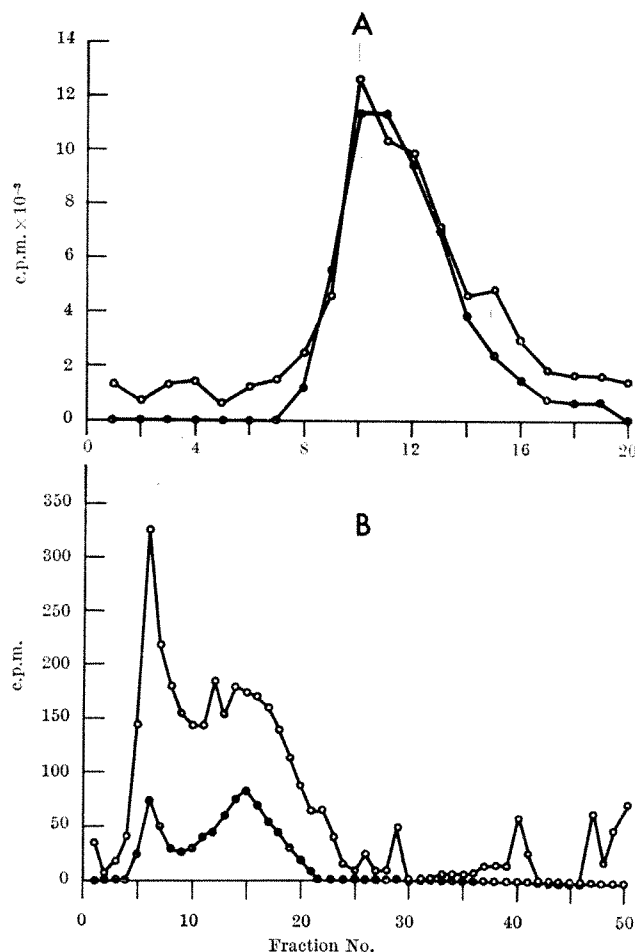


Fig. 1. Sucrose gradient analysis of RSV labelled with ^3H -thymidine and ^{14}C -uridine. RSV-infected chick embryo cells were exposed in culture to $50 \mu\text{Ci/ml}$ ^3H -thymidine (specific activity 20 Ci/mmol) and $0.1 \mu\text{Ci/ml}$ ^{14}C -uridine (50 mCi/mmol) overnight. The tissue culture conditions and purification of RSV were performed as described in Bishop *et al.*³. A, Isopycnic centrifugation in a 25–65 per cent sucrose gradient at 25,000 r.p.m. for 16 h at 4°C in a Spinco '25'3' rotor. B, Rate zonal centrifugation in a 15–30 per cent sucrose gradient with a 65 per cent sucrose cushion at 40,000 r.p.m. for 40 min at 4°C in a Spinco 'SW 41' rotor. Gradient fractions were collected and counted as described in Bishop *et al.*³. \circ , ^3H -thymidine; \bullet , ^{14}C -uridine.

rate zonal (Fig. 1B) centrifugation. Materials labelled with ^3H -thymidine and ^{14}C -uridine were found to sediment together. Biological activity as determined by the focus forming assay coincides with the peaks of radioactivity. Much of the rapidly sedimenting virus which reaches the 65 per cent sucrose cushion represents aggregates of RSV. The more slowly sedimenting peak has the rather broad distribution typical of RSV in rate zonal sucrose gradients. Biological activity again coincided with peaks of radioactivity. Subsequent experiments revealed that the Bryan high titre strain of RSV also incorporates ^3H -thymidine into the virion.

The RSV-associated thymidine was further studied by extracting the nucleic acid of the ^3H -thymidine labelled RSV according to the procedure of Bishop *et al.*³ and treating with deoxyribonuclease, ribonuclease and 1 M NaOH. The data in Table 1 indicate that the ^3H -thymidine labelled nucleic acid is sensitive to deoxyribonuclease but not to ribonuclease or 1 M NaOH. It seems likely therefore that the thymidine is contained in DNA rather than, for example, in transfer RNA. Because it is the methyl group of the thymidine that is labelled, the incorporation cannot be the result of demethylation and subsequent incorporation of uridine.

Could this putative piece of DNA be an artefact from the adsorption of cellular DNA onto the surface of the

Table 1. EFFECT OF NUCLEASES AND NaOH ON ^3H -THYMIDINE LABELLED NUCLEIC ACID FROM RSV

Treatment	^3H -Thymidine (c.p.m.)	Percentage degraded
None	2,651	
Deoxyribonuclease	734	72
None	2,734	
Ribonuclease	2,232	18
None	2,860	
NaOH	2,325	19

^3H -thymidine-labelled nucleic acid from RSV was prepared according to the procedures described in Fig. 2 and treated as follows: deoxyribonuclease (ribonuclease-free), $20 \mu\text{g/ml}$ in 0.01 M NaCl– 0.01 M Tris–HCl ($\text{pH } 7.4$)– 0.01 M MgCl_2 ; ribonuclease (boiled) $25 \mu\text{g/ml}$ in 0.01 M NaCl– 0.01 M Tris–HCl ($\text{pH } 7.4$)– 1 M NaOH. All preparations were incubated at 37°C for 1 h then precipitated with 10 per cent trichloroacetic acid and counted as described in Bishop *et al.*³.

virion? We tested this possibility in two ways. First, two reconstruction experiments were performed; one in which ^3H -thymidine-labelled uninfected chick embryo cells were sonicated and the sonicate was mixed with unlabelled RSV, and the other in which the growth medium from ^3H -thymidine-labelled uninfected chick embryo cells was mixed with unlabelled RSV. The mixtures were then purified according to the modification of the Duesberg procedure for RSV used in this laboratory³. No ^3H -thymidine counts were associated with the purified RSV in either preparation. The second set of experiments was designed to determine the constancy of the ratios of ^3H -thymidine to ^{14}C -uridine to focus forming units in different purified preparations. There is good correlation of the three parameters among the RSV preparations (Table 2). It therefore seems unlikely that the ^3H -thymidine in RSV is associated non-specifically.

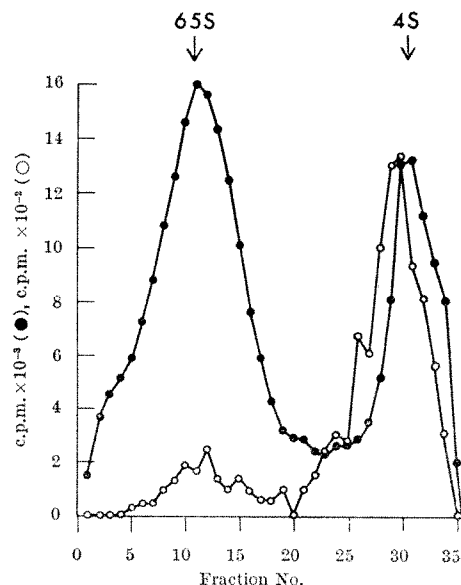


Fig. 2. Sucrose gradient analysis of ^3H -thymidine labelled nucleic acid from RSV. Labelled RSV was prepared by exposing infected cultures to $50 \mu\text{Ci/ml}$ ^3H -thymidine overnight. Similar cultures were exposed to $100 \mu\text{Ci/ml}$ $\text{H}_3^{32}\text{PO}_4$ overnight. The tissue culture conditions, purification of the RSV and extraction of the nucleic acid were as described in Bishop *et al.*³. A mixture of the ^3H -thymidine-labelled nucleic acid and the ^{32}P -labelled marker nucleic acid was centrifuged on a 15–30 per cent sucrose gradient at 60,000 r.p.m. for 75 min in the Spinco 'SW 65' rotor. Gradient fractions were collected and counted as described in Bishop *et al.*³. \circ , ^3H -thymidine; \bullet , ^{32}P .

From the data in Table 2, the estimated ratio of the number of thymidine:uridine molecules in the virion is 1:200. This value is at variance with the data of Robinson² which suggest that the amount of ^{32}P -labelled DNA in the nucleic acid recovered from RSV is less than 1 part in 2,000.

Experiments were then performed to characterize the RSV-associated DNA. To determine whether it was

Table 2. RELATIONSHIP OF ³H-THYMIDINE, ¹⁴C-URIDINE AND INFECTIVITY IN RSV PREPARATIONS

	¹⁴ C-U	c.p.m. ³ H-T	Infectivity (i.f.u.)	³ H/f.f.u.	Ratios ³ H/ ¹⁴ C	¹⁴ C/f.f.u.
I	404	188	2.7 × 10 ⁶	7.0 × 10 ⁻⁴	0.47	1.5 × 10 ⁻⁴
II	224	126	2.2 × 10 ⁶	5.8 × 10 ⁻⁴	0.57	1.0 × 10 ⁻⁴
III	680	616	1.0 × 10 ⁷	6.2 × 10 ⁻⁴	0.91	6.8 × 10 ⁻⁵

Doubly labelled RSV was prepared as described in Fig. 1 and the nucleic acids were extracted as described in Bishop *et al.*³. Infectivity assays were performed as described in Levinson⁴, and are expressed as focus forming units (f.f.u.)/ml.

located on the surface of the virion, intact RSV labelled with ³H-thymidine was treated with deoxyribonuclease. The data in Table 3 demonstrate that the bulk of the DNA is located on the interior of the particle or is protected in some other manner from degradation by deoxyribonuclease.

In addition, we examined both the size of the DNA and its relationship to the RNA of the virion. In rate zonal centrifugation in a 15–30 per cent sucrose gradient, using ³²P-labelled SR-RSV RNA as a marker, the bulk of

min) preparations of ³H-thymidine labelled nucleic acid from RSV were analysed by acrylamide gel (2.25 per cent) electrophoresis according to the procedure of Bishop *et al.*³. As shown by Duesberg⁶ and by Montagnier *et al.*⁷, heating the RNA in this manner causes the 65S RNA to dissociate into 35S sub-units.

Fig. 3A demonstrates that, on electrophoresis, the unheated DNA migrates in two principal bands in the vicinity of the 65S RNA marker. The 4S viral RNA marker can also be seen. Analyses of the heated mixture (Fig. 3B) shows that the 65S RNA marker has dissociated and now migrates in a heterodisperse fashion at approximately 30–40S (refs. 6, 7) but the bulk of the DNA migrates similarly to the unheated DNA. Because there is no indication that a significant amount of ³H-thymidine migrates with the sub-units of RSV-RNA, it is unlikely that most of the DNA is covalently bonded to the 65S viral RNA.

We conclude that the DNA we have characterized is not an artefact of the adsorption of cellular DNA to the virion; it is probably located in the interior of the particle.

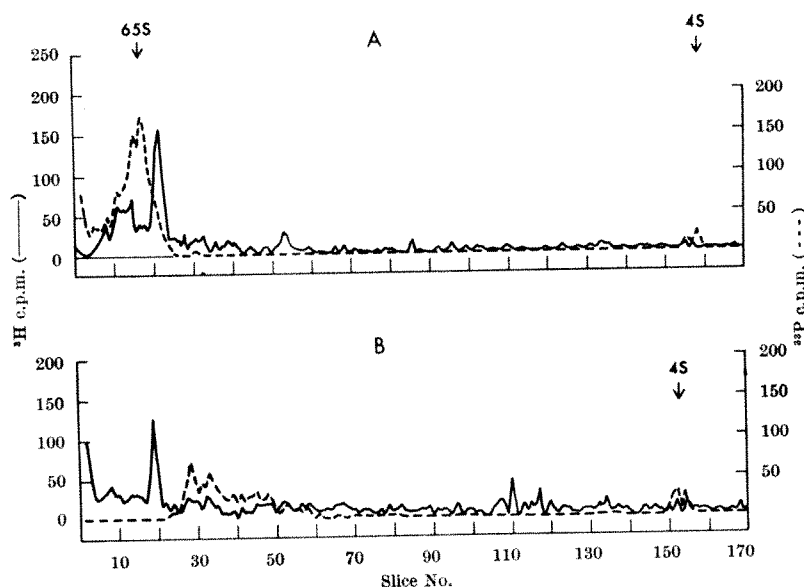


Fig. 3. Acrylamide gel electrophoretic analysis of unheated and heated ³H-thymidine-labelled nucleic acid from RSV. ³H-thymidine and ³²P-labelled nucleic acids from RSV were prepared as described in Fig. 2. A mixture of these nucleic acids was electrophoresed on 17 cm, 2.25 per cent acrylamide gels for 4–7 h at room temperature. A, Unheated; B, heated at 80° C for 1 min. —, ³H-thymidine; ---, ³²P.

the DNA sediments slightly ahead of the 4S component of the RSV-RNA (Fig. 2). A small amount of DNA co-sediments with the 65S genome RNA. It is of great interest that the two components of the DNA obtained from the virion sediment at approximately the same rate as the two DNA products synthesized *in vitro* by the recently identified virion RNA-dependent DNA polymerase (manuscript in preparation).

To determine whether some of the DNA was covalently bonded to the 65S RNA, unheated and heated (80° C, 1

The virion DNA may be the result of abortive synthesis during the maturation of the virus, but it could possibly represent either the template for a putative DNA-dependent DNA polymerase or cellular DNA incorporated into the virion during maturation.

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Table 3. EFFECT OF DEOXYRIBONUCLEASE ON THE INTACT RSV PARTICLE LABELLED WITH ³H-THYMIDINE

	No sonication	³ H-Thymidine (c.p.m.) Percentage degraded	Sonication	Percentage degraded
Without	206		198	
With	157	24	134	32

³H-thymidine-labelled RSV was prepared as described in Fig. 2 and treated with deoxyribonuclease (ribonuclease-free) 100 μg/ml. in Mg²⁺ buffer as described in Table 1. One sample of RSV was sonicated for 30 s using a Bronwill sonicator at power setting 1 before enzyme treatment.

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Formation of Viral RNA-DNA Hybrid Molecules by the DNA Polymerase of Sarcoma-Leukaemia Viruses

by

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Viral RNA is the template for the DNA polymerase of the sarcoma-leukaemia viruses: a "natural" RNA-DNA hybrid is formed by the viral DNA polymerase, and a "synthetic" RNA-DNA hybrid is formed by annealing viral RNA with the DNA product of the DNA polymerase reaction.

WE have postulated that RNA tumour viruses possess a DNA polymerase dependent on viral RNA (ref. 1) and several laboratories have recently reported that leukaemia and sarcoma viruses of the mouse, chicken and cat incorporate radioactive deoxyribonucleotides into an acid-insoluble form²⁻⁵. As well as showing that information can flow from RNA to DNA, this system may provide unique information for the understanding of carcinogenesis. Although it is clear that DNA is the reaction product, the evidence that viral RNA is the template has been indirect; pretreatment of virus particles with large amounts of ribonuclease inhibits subsequent enzyme activity; RNA tumour viruses contain a 70S RNA component but little DNA.

We describe here two lines of evidence demonstrating that viral RNA is the template for the murine sarcoma virus (MSV) DNA polymerase: first, viral RNA-DNA hybrid molecules are formed by the MSV DNA polymerase and second, the DNA product formed by the MSV DNA polymerase hybridizes with viral RNA.

Viral RNA-DNA Hybrids

We have characterized previously the DNA polymerase present in MSV (ref. 4). The standard assay is conducted in a final volume of 100 μ l. containing 40 mM Tris buffer (pH 8.1), 30 mM NaCl, 5 mM dithiothreitol, 2.5 mM $MgCl_2$, 0.01 per cent 'Nonidet P40', 0.1 mM dATP, dGTP, dCTP, and 10 μ Ci of 3H -TTP (11.4 Ci/mmol). Enzyme activity

is linear with time for at least 90 min and is proportional to virus concentration from at least 2 to 20 μ g of viral protein per 100 μ l. All four deoxyribonucleotides are incorporated into an acid-insoluble, alkali-stable, deoxy-ribonuclease-digestible product.

To determine whether a viral RNA-DNA hybrid is synthesized by the viral DNA polymerase, MSV(M) containing ^{32}P -labelled viral RNA was used in the standard enzyme assay with 3H -TTP as the labelled deoxyribonucleotide precursor of DNA. The reaction was stopped after 0, 15, 30, 60, and 90 min and the products were analysed by zone sedimentation (Fig. 1). In the conditions used, ^{32}P -labelled viral RNA sediments about two-thirds down the tube. No significant amount of 3H -labelled material from the zero time reaction sediments in this 70S region, but products of increasing times of reaction include increasing amounts of 3H -DNA co-sedimenting with 70S RNA. At 90 min, more than 8,000 c.p.m. of the 3H -DNA product from 50 μ l. of reaction mixture co-sediments with 70S RNA.

The peak fraction containing both 70S ^{32}P -RNA and 3H -DNA were pooled as a "hybrid fraction" for further study. The hybrid fraction was treated with pronase (125 μ g/ml. for 1 h at 37°C) and recentrifuged in a sucrose density gradient. Again the 3H -DNA and ^{32}P -RNA sedimented in the same 70S position, indicating that the DNA and RNA were not held together by a protein link. Stronger evidence for the hybrid nature of the product

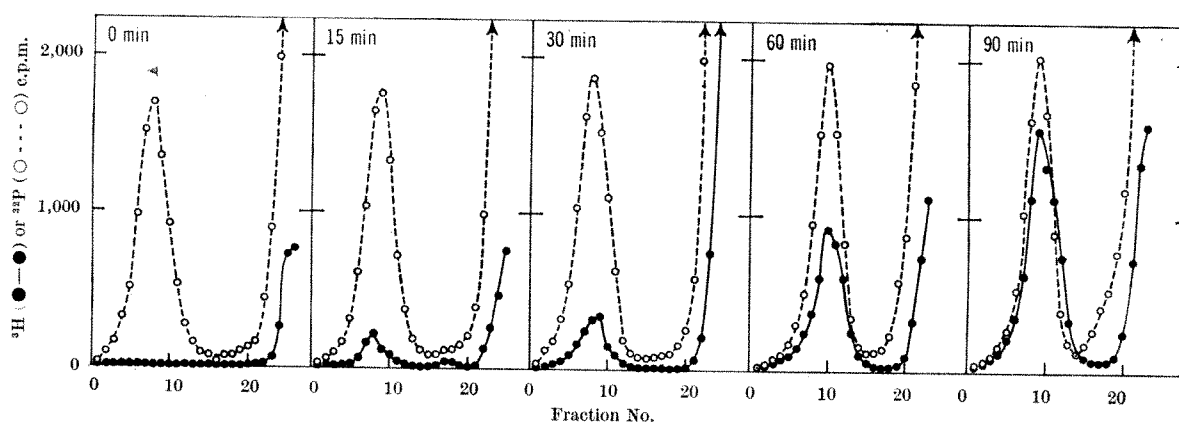


Fig. 1. Zone sedimentation of the product of the MSV DNA polymerase reaction. The Moloney isolate of murine sarcoma virus, MSV(M), purified from the growth medium of the MSV(M) transformed rat cell line, 78A1⁴, was used in these experiments. ^{32}P -labelled MSV(M) was purified from the media of fifty 78A1 monolayer cultures (250 ml. Falcon plastic) grown for 24 h in Eagle's minimal essential medium (containing one-fourth the normal phosphate concentration) with 10 per cent foetal calf serum containing 50 μ Ci/ml. of carrier-free ^{32}P . Purified virus was dialysed for 3 h against 0.01 M Tris-HCl (pH 8.1). Five 200 μ l. amounts of virus (34.5 μ g of protein) were made up to 500 μ l. containing the standard assay mixture with 50 μ Ci of 3H -TTP. At 0, 15, 30, 60 and 90 min of incubation at 37°C, one assay tube was removed, and the reaction stopped by the addition of 10 mM EDTA and 0.5 per cent SDS. The preparations were layered over 11.4 ml. linear 15–30 per cent sucrose gradients in 0.01 M Tris-HCl (pH 7.4)—0.1 M NaCl—0.001 M EDTA, and centrifuged at 4°C for 3.5 h in the Spinco 'SW41' rotor at 36,000 r.p.m. Fractions (0.5 ml.) were collected and 50 μ l. aliquots plus 100 μ g of carrier calf thymus DNA were precipitated with 0.6 M trichloroacetic acid and collected on membrane filters. The acid-insoluble 3H and ^{32}P c.p.m. were determined in a Beckman liquid scintillation spectrometer. (^{32}P c.p.m. at the top of the gradient represent viral envelope phospholipid and 4–5S RNA present in tumour viruses. 3H c.p.m. at the top are mostly 3H -TTP from the assay mixture and also some free 3H -DNA product.) ○—○, Viral ^{32}P -RNA; ●—●, 3H -DNA product.

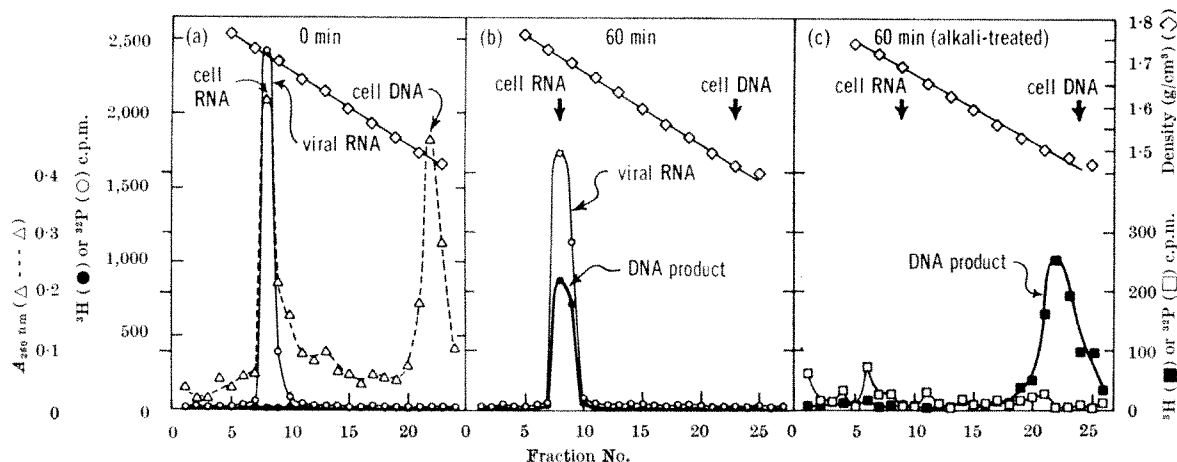


Fig. 2. Equilibrium sedimentation of the viral RNA-DNA hybrid fractions in Cs_2SO_4 density gradients. The peak fractions containing ^3H -DNA and/or viral ^{32}P -RNA after zone sedimentation of the 0 and 60 min reaction products (Fig. 1) were pooled and dialysed against $0.1 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M NaCl}-0.015 \text{ M sodium citrate}$). Two hundred μl . of hybrid and 20 μg each of mouse cell DNA and RNA (absorbance markers) were made up to a final volume of 3.1 ml. in $2 \times \text{SSC}$, 2.60 g of Cs_2SO_4 (Harshaw Chemical Co.) were added, and the tubes were centrifuged at 20°C for 84 h in the Spinco 'SW39' rotor at 33,000 r.p.m. Fractions (0.17 ml.) were collected and the refractive index, absorbance at 260 nm, and acid-precipitable radioactivity determined.

was obtained using equilibrium band sedimentation in a Cs_2SO_4 gradient which resolves DNA from RNA by density. As shown in Fig. 2a, the 70S ^{32}P -RNA band is at a density close to that of the cell RNA marker ($\rho \approx 1.68 \text{ g cm}^{-3}$) and distinct from that of the cell DNA marker ($\rho \approx 1.44 \text{ g cm}^{-3}$). But the 60 min ^3H -DNA band is coincident with viral ^{32}P -RNA (Fig. 2b). Treatment of the hybrid fraction with 0.2 M NaOH for 20 min at 80°C before density gradient analysis—which would hydrolyse RNA—lowers the density of the ^3H -DNA component to that of free DNA (Fig. 2c). Three additional hybrid fractions were analysed with similar results. We conclude: (1) that the immediate product of the MSV DNA polymerase reaction is a viral RNA-DNA hybrid; and (2) that only a small portion of each RNA molecule seems to participate in hybrid duplexes, for the buoyant density of the hybrid is close to that of viral RNA.

The RNA- ^3H -DNA fractions isolated by zone sedimentation of reaction mixtures after 30, 60 and 90 min were sedimented through alkaline sucrose gradients which free polynucleotide chains from one another. After 10 h at 36,000 r.p.m. in the 'SW41' rotor (Fig. 3), ^{14}C -DNA released from marker adenovirus sediments at 34S near the bottom of the tube. The alkali-released ^3H -DNA from the hybrid sediments close to 7S in six determinations. This corresponds to a molecular weight of single-stranded DNA of about 200,000 daltons (Table 1).

Table 1. SIZE OF DNA IN VIRAL RNA-DNA HYBRID

Incubation (min)	Sedimentation coefficient in alkali*	Mol. wt.†
30	6.3	155,000
30	5.9	150,000
60	6.7	181,000
60	6.7	181,000
90	7.7	255,000
90	6.8	188,000

* Sedimentation coefficient in alkali determined in Fig. 3.

† Molecular weight of single stranded DNA calculated from S value as described by Studier⁴.

Further evidence that viral RNA is the template for the MSV DNA polymerase was obtained by annealing viral 70S ^{32}P -RNA with enzymatically synthesized ^3H -DNA and analysing the product by equilibrium sedimentation in Cs_2SO_4 density gradients. When viral RNA and DNA are mixed but not annealed, the viral DNA band is at the density of unhybridized DNA (Fig. 4a). But the incubation of viral ^{32}P -RNA with ^3H -DNA in annealing conditions converts viral DNA to a hybrid—it again coincides with viral RNA (Fig. 4b).

Does the Virus contain DNA?

RNA tumour viruses are assumed to contain only RNA but because DNA might serve as a template for the viral DNA polymerase, we attempted to determine whether MSV(M) contains appreciable amounts of DNA. We isolated MSV(M) from the media of 78A1 cells grown for 24 h in the presence of 2 mCi of ^3H -thymidine; the purified preparation represented 2.21 mg of protein and 41,840 c.p.m. (^3H). Thus less than 120,000 daltons of DNA are present per virus particle (on the basis of the specific radioactivity of newly synthesized cell DNA and an assumed virus particle content of 60 per cent protein and 2 per cent RNA and a particle weight of 450×10^6 daltons^{7,8}).

MSV (^3H -thymidine pre-labelled) was used in the standard enzyme assay with ^{32}P -TTP as the labelled deoxyribonucleotide precursor of DNA. The reaction mixture was treated with 0.5 per cent sodium dodecyl sulphate and analysed by zone sedimentation. Most of the

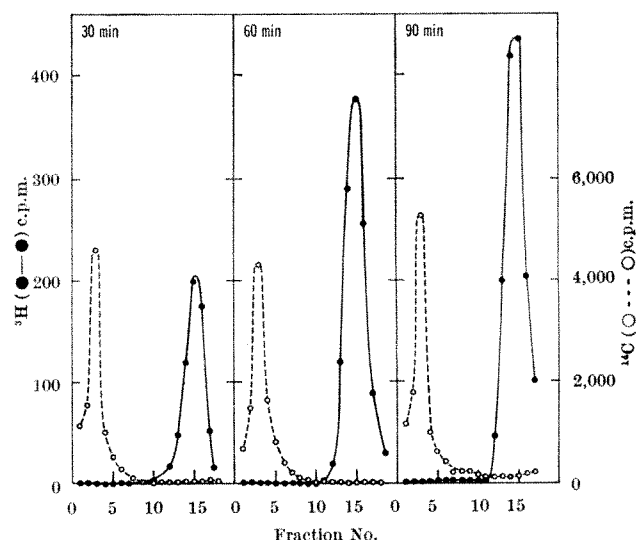


Fig. 3. Zone sedimentation of ^3H -DNA from RNA- ^3H -DNA hybrids in alkaline sucrose density gradients. The 30 min (200 μl), 60 min (150 μl), and 90 min (100 μl) hybrid fraction, isolated as described in Fig. 1, plus 3 μl . of ^{14}C -thymidine labelled adenovirus type 7 were layered on 11.3 ml. linear 5–20 per cent sucrose gradients in 0.3 M NaOH–0.7 M NaCl–0.001 M EDTA, and centrifuged at 4°C for 10 h in the Spinco 'SW41' rotor at 36,000 r.p.m. Fractions (0.75 ml.) were collected and the acid-insoluble, alkali-stable radioactivity was determined⁴. \circ — \circ , Adenovirus DNA marker; \bullet — \bullet , DNA product from RNA-DNA hybrid.

^{32}P -DNA product sedimented to the position of the RNA-DNA hybrid but ^3H c.p.m. from pre-labelled virus did not co-sediment with the DNA product. Thus the ^3H -thymidine-labelled material in MSV does not seem to participate in the formation of the ^3H -DNA-containing hybrid. The small amount of ^3H -thymidine present in the virus preparation may represent DNA in structures contaminating the virus preparation, or possibly the natural DNA product of the viral DNA polymerase.

Significance of the DNA Product

Our experiments strongly argue that viral RNA is the template for the DNA polymerase present in the murine sarcoma virus for (1) a "natural" viral RNA-DNA hybrid is formed during the enzymatic reaction, and (2) a "synthetic" RNA-DNA hybrid is formed by annealing the DNA product with viral RNA. The viral RNA-DNA hybrid is probably the initial product of the MSV DNA polymerase reaction for only small amounts of free DNA product are found even after 90 min of reaction.

produce hybrid structures longer than about 2-7 per cent of the viral genome would then have to be explained. Nuclease activities might degrade the rest of the chain formed *in vitro*, or perhaps host cell or viral induced gene products are required to transcribe the entire viral genome into DNA. The second alternative opens up several possibilities: this piece(s) of DNA may, for example, code for critical protein(s) in viral development. The distinction between these two possibilities is especially important, for the role of viral DNA appears, on the basis of inhibitor studies, to be essential for both viral replication and cell transformation¹. The mechanism of carcinogenesis by sarcoma-leukaemia viruses may even provide a model for possible reversal of RNA transcription during normal cell function, such as the intriguing expression of RNA tumour virus genetic information by cellular genes during embryonic development in the mouse¹⁰.

We thank Miss Mary Beranek, Miss Maria Cartas, and Mrs Clara Boudreau for assistance and Dr David

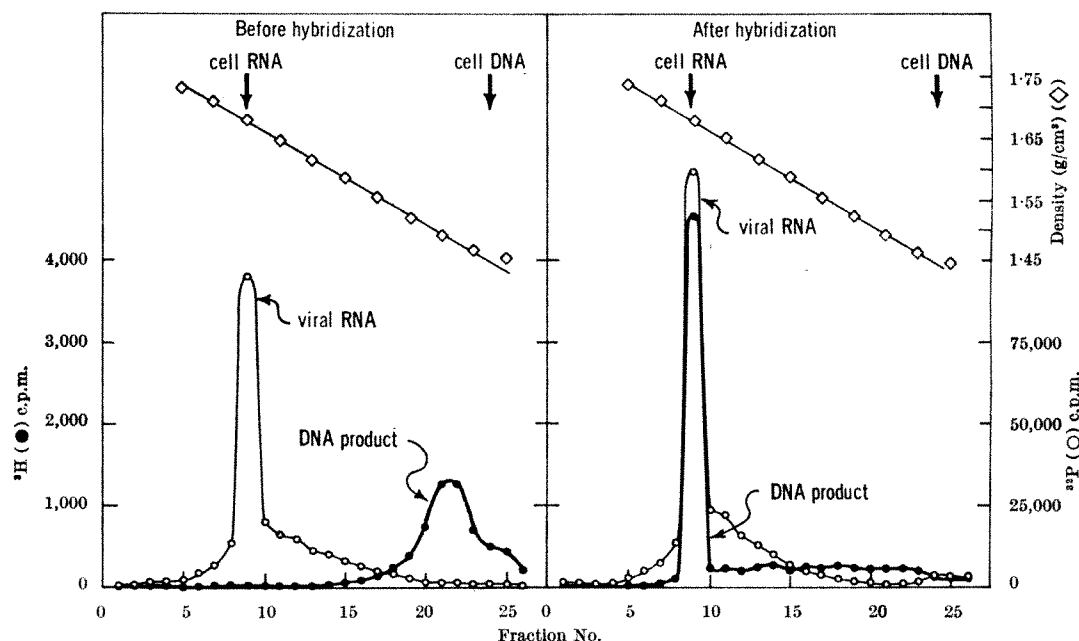


Fig. 4. Hybridization of viral ^{32}P -RNA with the ^3H -DNA product of the MSV DNA polymerase. Viral ^{32}P -RNA was prepared from ^{32}P -labelled MSV(M) and ^3H -DNA was prepared from a 60 min reaction mixture as follows: after the addition of 0.5 per cent sodium dodecyl sulphate, the preparations were extracted three times with phenol saturated with 0.1 M NaCl-0.01 M Tris-HCl (pH 7.4)-0.001 M EDTA. The aqueous layer was dialysed against $0.1 \times \text{SSC}$. The ^3H -DNA preparation was treated with 0.2 N NaOH at 80°C for 20 min and neutralized to denature the DNA and destroy unlabelled viral RNA. Denatured ^3H -DNA (4,400 c.p.m.) and $5.8 \mu\text{g}$ of viral ^{32}P -RNA (40,500 c.p.m./ μg) in $400 \mu\text{l}$ of $2 \times \text{SSC}$ were annealed at 60°C for 9 h. An identical mixture of ^3H -DNA and ^{32}P -RNA, not annealed, served as a control. Both samples were centrifuged to equilibrium in Cs_2SO_4 gradients and processed as described in Fig. 2.

The DNA component in an individual hybrid duplex moves at 7S, corresponding to a molecular weight of 200,000. A similar value is obtained for the average amount of DNA formed per virus particle in a 90 min enzyme reaction (see Fig. 1, calculated from $3.45 \mu\text{g}$ of viral protein per $50 \mu\text{l}$ reaction mixture, 8,000 c.p.m. incorporated into DNA, assuming equimolar amounts of A, G, C and T in the DNA product and a 60 per cent protein content and 450×10^6 particle weight for the virus^{7,8}). These findings confirm the correspondence between the RNA-DNA hybrid and the free 70S viral RNA: apparently only a small fraction of each RNA molecule is in hybrid form.

Further experiments must resolve whether (1) the complete viral genome is transcribed to DNA *in vivo* or (2) the production of a specific short piece(s) of DNA is a true function of the polymerase. According to the first alternative, the hybrid may be an intermediate in the replication of virus or in the production of transforming DNA, but the failure of the *in vitro* enzyme reaction to

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DNA-directed DNA Polymerase Activity in Oncogenic RNA Viruses

by

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Besides having RNA-dependent DNA polymerase activity, oncogenic RNA viruses possess a DNA-directed DNA polymerase which is distinguished from previously described enzymes of this type in preferring double-stranded DNA as template and yielding a principally double-stranded product.

Temin¹ and Baltimore² recently reported the existence of an RNA-directed DNA polymerase in oncogenic RNA viruses. We confirmed these findings with six oncogenic viruses and further established³ the following features of the reaction: (a) physical and chemical characterizations proved that the product was in fact a DNA heteropolymer; (b) molecular hybridization showed that the DNA synthesized was complementary to the viral RNA contained in the enzyme preparation; and (c) RNA-DNA complexes were detected as early components in the polymerization. The specific complementarity of the synthetic DNA to viral RNA and the early appearance of RNA-DNA hybrids implied that the viral RNA functioned as a template in the synthesis of the DNA.

The heritably stable state that characterizes cells transformed by these oncogenic agents seems to require integration of the newly synthesized DNA into the genome of the cell. It is unlikely that the RNA-DNA hybrids detected as presumptive intermediates in the reaction can serve this purpose. It is more likely that the single-stranded DNA has to be converted into its double-stranded equivalent for integration, a conversion that would necessitate a DNA-directed DNA polymerase. We therefore pointed³ to the need to search for such an enzyme, either in the virion or in the infected cell, which would presumably be characterized by its ability to use duplexes to generate duplexes. We have now examined six oncogenic RNA viruses—Rauscher leukaemia virus (RLV), Rous sarcoma virus RSV-(RAV-1), avian myeloblastosis virus (AMV), murine mammary tumour virus (MTV), Moloney sarcoma virus (MSV) and the feline leukaemia virus (FeLV)—and encountered a DNA-directed DNA polymerase in the virions of all of them. We report here some of the properties of the reactions and the products formed.

Previously³, we alluded to two observations in prolonged syntheses that could not be explained solely by the RNA-directed DNA polymerase activity we were studying. The base composition of the product of an extensive synthesis seemed to be more identical than complementary to the viral RNA. This implied that the DNA complement made early in the reaction was subsequently used as a template for the formation of a complement to itself. In addition, although early products are all hybridizable, late DNA components appear that do not complex to viral RNA (unpublished observations). The implication is that these late DNA products are either duplexes or single strands identical in sequence to the viral RNA. All this suggests the presence of a DNA-directed DNA synthesis in which the DNA formed initially serves as a template for subsequent polymerization.

Evidence for a DNA-directed DNA Polymerase

In the experiment documented in Table 1, the addition of either *Escherichia coli* DNA or mouse embryo fibroblast

(MEF) DNA results in a striking stimulation of ³H-dATP incorporation. A trivial explanation of this would invoke protection by the added DNA against nucleolytic degradation of the DNA synthesized by the RNA-directed polymerase. To test—and eliminate—this, and at the same time provide a more convenient system for studying the DNA-directed step, it was necessary to eliminate the RNA-directed activity. This can be done by destroying the resident viral RNA by previous treatment of the disrupted virions with a suitable nuclease. Micrococcal nuclease is convenient because it requires Ca²⁺. Its activity can consequently be readily neutralized by the specific chelating agent ethyleneglycol-bis-(aminoethyl ether)tetraacetic acid (EGTA). Fig. 1 shows the kinetics of DNA synthesis in four oncogenic viral preparations that had been pretreated with micrococcal enzyme. There is little residual activity in the absence of added exogenous nucleic acid; the addition of DNA leads to an excellent response in all four enzyme preparations.

Table 2 shows that the DNA-stimulated reaction requires all four deoxyriboside triphosphates as well as Mg²⁺. The last control recorded in Table 2, omission of the viral enzyme, was included to eliminate the unlikely possibility that the enzyme we were detecting was present as a contaminant of the micrococcal nuclease used to eradicate the RNA-directed step.

Table 1. STIMULATION OF THE AMV POLYMERASE BY DNA

DNA	-DNA	+DNA
	c.p.m.	
<i>E. coli</i> (4.0 µg)	631	3,460
MEF (1.5 µg)	840	4,340

A standard incubation mixture of 1 ml. contains in µmoles: 50 Tris-HCl (pH 8.3), 12 MgCl₂, 40 KCl, 2 dithiothreitol, 0.8 each of the non-labelled deoxyribonucleoside triphosphates and 0.04 labelled deoxyribonucleotide. In this experiment, ³H-dATP was used at a specific activity of 330 c.p.m. The incorporations represent those observed in 0.1 ml. aliquots, corresponding to 15 µg viral protein. Virus particles suspended in 0.01 M Tris (pH 8.3) at 300 µg viral protein/ml. were preincubated 10 min at 0° C in the presence of 0.2 per cent 'Nonidet P-40' detergent with dithiothreitol at 30 mM. The virus was then added to a standard incubation mixture at a level of 130 µg/ml. and incubated at 37° C for 30 min. The reaction was terminated by the addition of 0.5 ml. water and 0.3 ml. trichloroacetic acid (TCA) mixture (equal volume mixture of 100 per cent TCA solution, saturated sodium orthophosphate, and saturated sodium pyrophosphate). After 10 min, the precipitable radioactivity was collected on a nitrocellulose filter, dried, and the radioactivity determined in a liquid scintillation counter using BBOT-toluene scintillation fluid. Mouse embryo fibroblast DNA and *E. coli* DNA were extracted following the procedure of Gillespie and S.S. and added to the reaction mixtures as indicated.

Table 2. REQUIREMENTS OF DNA-DIRECTED DNA POLYMERASE

Conditions	³ H-dAMP incorporated (c.p.m.)
Complete (12 mM Mg ²⁺ , 40 mM K ⁺)	
1 µg MEF DNA	404
-dCTP	55
-dGTP	15
-dATP	41
-Mg ²⁺	62
-Viral polymerase	30

Enzyme reactions were carried out as described in Table 1. The virus suspension was treated with micrococcal nuclease before incubation at 37° C, as described in Fig. 1. The specific activity of the ³H-dATP was 330 c.p.m./pmole.

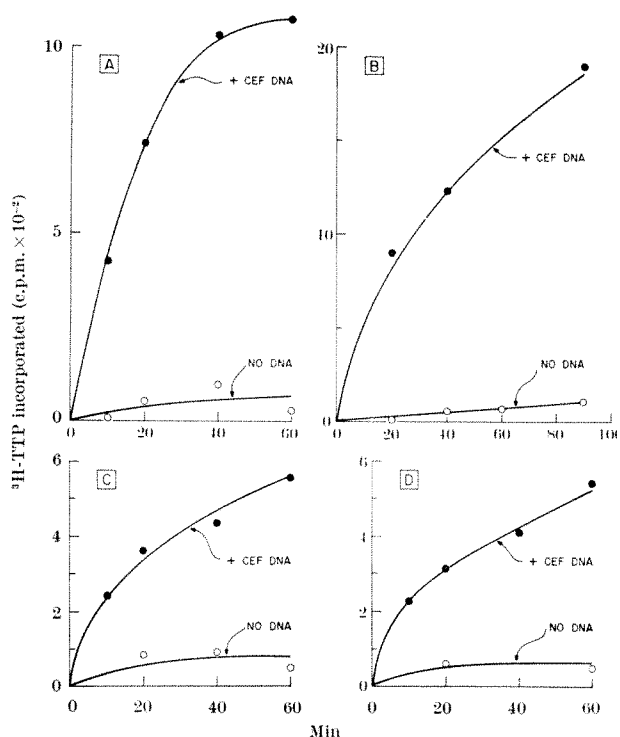


Fig. 1. Kinetics of incorporation of ^3H -TTP by DNA-directed DNA polymerases from (A) RSV-(RAV-1), (B) AMV, (C) RLV and (D) MTM. For each reaction, a 0.25 ml. of a standard reaction mixture (Table 1) was used. The specific radioactivity of ^3H -TTP was 1.4×10^5 c.p.m./pmole. Chick embryo fibroblast DNA at a concentration of $3 \mu\text{g}/0.25$ ml. reaction was used as template for the four DNA-DNA polymerases. The DNA was prepared as described previously⁷ from trypsinized chick embryos. The preparation, source, and purification of the viruses used have been described previously. To eliminate the RNA-directed reaction, all the virus preparations used were submitted to a non-ionic detergent treatment ('NP-40', Shell Co.) for 10 min at 0°C in the presence of 0.1 M dithiothreitol in volumes of about $25 \mu\text{l}$. The 'NP-40' concentrations were 0.2 per cent for RSV-(RAV-1) and AMV, 0.1 per cent for RLV, and 0.17 per cent for MTM. These were then diluted to $250 \mu\text{l}$ for digestion with nuclease from *Staphylococcus aureus* (Worthington); incubation was at room temperature for 30 min in the presence of $240 \mu\text{g}$ nuclease/ml. and 0.002 M Ca^{2+} . The nuclease action was stopped by adding EGTA to a final concentration of 0.004 M. Nucleoside triphosphates were then added and incorporation kinetics followed at 37°C . The amounts of viral protein per $30 \mu\text{l}$: A, $3.75 \mu\text{g}$; B, $3.90 \mu\text{g}$; C, $2.56 \mu\text{g}$ and D, $2.80 \mu\text{g}$. At the indicated times, $30 \mu\text{l}$ aliquots were withdrawn, precipitated with TCA in the presence of $60 \mu\text{g}$ of *E. coli* RNA as carrier, processed and counted as described in Table 1.

Nature of the Product

The product of an extensive synthesis was purified by phenol extraction and alcohol precipitation. It was then subjected to physical and enzymological tests. Table 3 shows that the acid-precipitable product can be degraded by deoxyribonuclease and spleen phosphodiesterase but not by ribonuclease or alkali. To confirm that it is DNA, the density of the product was examined by equilibrium density centrifugation in Cs_2SO_4 and the result is shown in Fig. 2. *E. coli* DNA was included as a density marker (1.426 g cm^{-3}). The density of the DNA synthesized in response to the addition of MEF DNA is found at the same density (1.420) as MEF DNA. The DNA produced by the RNA-directed reaction has³ the indicated density of 1.450.

The average size of the product is compared (Fig. 3) with that of template in neutral and alkaline sucrose gradients. It is clear that the radioactive product is smaller than the DNA used to stimulate the reaction; there is no evidence of covalent attachment of the product to the template.

Response to Various DNA Templates

Table 4 compares the response of the AMV DNA-DNA polymerase to different DNA templates. Unlike the thoroughly studied DNA-dependent DNA polymerase

Table 3. PROPERTIES OF DNA-DIRECTED PRODUCT OF THE AMV POLYMERASE

Treatment	TCA precipitable c.p.m.	Per cent
None	810	100
Ribonuclease (50 $\mu\text{g}/\text{ml}$.)	785	97
KOH (0.33 M, 18 h)	773	95
Deoxyribonuclease (120 $\mu\text{g}/\text{ml}$.)	177	22
Spleen phosphodiesterase (0.28 units/ml.)	14	1.7

The product DNA made on double-stranded MEF DNA template was synthesized using the standard incubation mixture on a 1 ml. scale. The specific activity of the ^3H -ATP used was 4,000 c.p.m./pmole. After reaction at 37°C for 2 h, the product was isolated by making the reaction mixture 0.4 M with respect to NaCl and 1 per cent sodium dodecyl sulphate (SDS) and extracting for 5 min at room temperature with an equal volume of a mixture of phenol-cresol (10:1). The aqueous phase was filtered through a 'Sephadex G-50' column (0.9×100 cm) and the purified material precipitated with alcohol. It was then dissolved in 0.01 M Tris- 0.1 M NaCl (pH 8.3) (TN buffer) and portions of this solution were used for the different experiments. The digestion with ribonuclease was carried out in 0.5 ml. TN buffer for 90 min at 37°C . Digestion with deoxyribonuclease was for 3 h at 37°C in TN buffer plus 0.005 M MgCl_2 . The reaction with spleen phosphodiesterase was in 0.02 M ammonium acetate buffer (pH 6.0) at 37°C for 2.5 h in 0.1 ml. The alkali treatment was in $20 \mu\text{l}$ in 0.33 M KOH for 18 h at 37°C .

of Kornberg⁴, these oncogenic viral DNA polymerases prefer double to single-stranded DNA. A particularly interesting example is the f1 DNA, a single strand DNA quite similar to that found in the DNA bacteriophage ϕX174 which has been shown⁵ to be an excellent template for the Kornberg enzyme. We have seen no signs of template function even in extensive incubations with f1 DNA. The f1-RF DNA, a covalently linked double-stranded circular DNA, is also inactive. This is not surprising because at least one of the two strands would have to be nicked before replication could commence.

Table 4. RESPONSE OF AMV DNA-DNA POLYMERASE TO VARIOUS DNA TEMPLATES

DNA	c.p.m. ^3H -TTP incorporated	Double-stranded	Single-stranded or denatured
<i>E. coli</i>	—	1,006	239
MEF	135	638	135
CEF	76	889	403
T6	120	2,320	460
f1	50	—	19
f1-RF	50	128	—

The reactions were in the standard conditions and processed as in Table 1. The ^3H -dATP had a specific activity of 350 c.p.m./pmole. Incubations were for 30 min at 37°C . DNA concentrations were about $3 \mu\text{g}/0.25$ ml.

Proof by Hybridization

The response to quite disparate DNA templates provides an excellent opportunity of deciding by molecular hybridization whether the DNA added is in fact serving as an instructive agent in the polymerization. The required DNA-DNA hybridization can readily be performed using the Denhardt⁶ modification of the Gillespie and Spiegelman⁷ method for hybridizing RNA to DNA fixed to membrane filters. We have found (Horowitz and S.S., unpublished observations) that including the

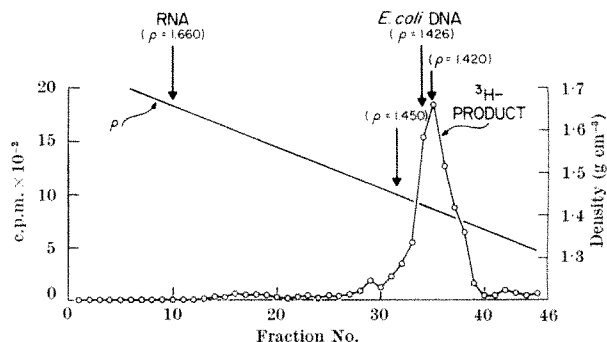


Fig. 2. Caesium sulphate equilibrium density gradient centrifugation of AMV DNA-DNA polymerase product. A 1 ml. standard reaction (Table 1) containing $133 \mu\text{g}$ viral protein, $9 \mu\text{g}$ MEF DNA, and ^3H -dATP at 4.1×10^5 c.p.m./pmole was incubated for 2 h at 37°C . The reaction mixture was deproteinized by phenol extraction at room temperature in the presence of 0.4 M NaCl and SDS 1 per cent final concentration. An aliquot of the purified product was mixed with saturated Cs_2SO_4 to a density of 1.550 and centrifuged at $33,000$ r.p.m. at 20°C for 60 h in a Spinco 'SW-56' rotor. *E. coli* DNA ($\rho = 1.426$) and ^{32}P -labelled 18S ribosomal RNA ($\rho = 1.660$) were used as internal markers. After centrifugation, fractions were collected from the bottom of the tube and processed as in Table 1.

alkaline wash used in the procedure of Warnaar and Cohen⁸ yields acceptable backgrounds.

Table 5 shows the results of hybridizing each of three DNA products to the three DNA templates used in the synthetic reactions. The data are clear cut: the hybridizability of each product is much superior when challenged with the DNA actually used in its synthesis. The percentages of the input counts hybridized in the homologous hybridizations of Table 5 are quite considerable: 10, 16 and 59 per cent for the mouse, *E. coli* and T6, respectively. For the mouse the value is surprisingly high in view of the complexity of the genome. We have obtained similar results with chicken DNA. Such data suggest that the DNA polymerase of the oncogenic viruses is not copying random segments of these vertebrate DNAs. Clearly it would be desirable to identify the components of the genome that are being chosen selectively for amplification.

Another feature of the hybridization experiments is exhibited in Table 6 which examines the effect of denaturation on the ability of the synthesized DBA to hybridize to its homologous templates. Denaturation strikingly augments the amount of product available for hybrid formation. The results suggest therefore that much (70–80 per cent) of the DNA synthesized is in the double-stranded state.

Conclusions

While eliminating the resident RNA template an opportunity is presented to examine the acceptability of other RNA molecules by the RNA-directed polymerase. Experiments along these lines will be reported later.

The fact that the DNA-directed polymerases described here readily accept—indeed prefer—double-stranded DNA as templates distinguishes them from the DNA-dependent DNA polymerases so far reported. If the oncogenic viral polymerases are in fact true replicases, they should

Table 5. HYBRIDIZATION OF DNA-DIRECTED PRODUCTS TO VARIOUS DNAs

DNA on filter	Mouse	<i>E. coli</i>	T6
Mouse	8,698 (100%)	587 (6%)	1,477 (2%)
<i>E. coli</i>	454 (5%)	7,724 (100%)	197 (0.4%)
T6	152 (2%)	168 (2%)	54,673 (100%)

Radioactive material in each hybridization was equivalent to 3.3×10^4 , 4.9×10^4 and 9.4×10^4 c.p.m. for DNAs synthesized with mouse, *E. coli*, and T6 DNA, respectively. The numbers represent c.p.m. found per 150 μ g of DNA on the filter; those in parentheses represent percentage of that observed in the homologous hybridization. Products were prepared as described in Table 3, and dissolved in 0.5 ml. of 0.07 \times SSC (1 \times SSC = 0.15 M NaCl–0.015 M sodium citrate, pH 7). The hybridization reactions were carried out using DNA immobilized on membrane filters and product DNA in solution in 200 μ l. of 3 \times SSC at 66° C for 12 h. For denaturation, 100 μ l. of the product was made 0.1 M with respect to KOH and kept at room temperature for 10 min. The solution was then neutralized with an equivalent amount of HCl and diluted to a final volume of 200 μ l. with a calculated amount of SSC solution so that the final solution was 3 \times SSC. The filters were loaded with 100–150 μ g of denatured DNA, allowed to dry at room temperature, and then transferred to a vacuum oven at 80° C for 2 h at a pressure of 25 mm Hg. The filters were pre-incubated in Denhardt's solution (0.02 per cent bovine serum albumin, 0.02 per cent Ficoll and 0.02 per cent polyvinylpyrrolidone in 3 \times SSC) at 66° C for 6 h immediately preceding hybridization. After hybridization for 12 h, the filters were taken out; each was washed on either side with 100 ml. of 10^{-3} Tris (pH 9.3), dried and counted. c.p.m. fixed in homologous hybridizations are italicized.

Table 6. HYBRIDIZATIONS OF NATIVE AND DENATURED PRODUCT DNA WITH TEMPLATE DNA

DNA	Native	Denatured
Mouse	2,878	8,698
<i>E. coli</i>	2,098	7,724
T6	10,834	54,673

The same products and conditions are used here as for the experiment of Table 5. The "native" was not, however, subjected to the alkaline treatment as in the legend of Table 5.

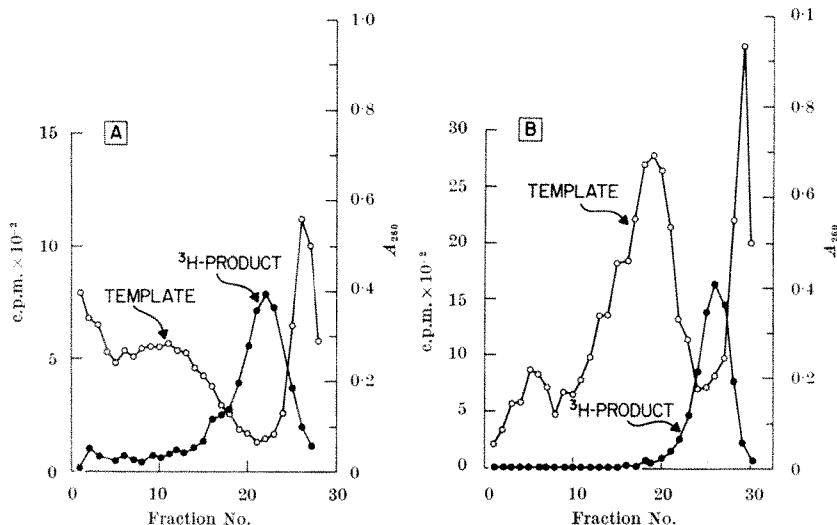


Fig. 3. Neutral and alkaline sucrose gradient centrifugation of AMV DNA-DNA polymerase product. A 1 ml. reaction containing 133 μ g of AMV protein pretreated as described in Fig. 1, 9 μ g of *E. coli* DNA, and ^3H -dATP (4×10^5 c.p.m./pmole) was incubated for 2 h at 37° C and the product purified as described in Fig. 2. 180 μ g of the *E. coli* DNA used as template and an aliquot of the product of the reaction were layered on (A) neutral (8–22 per cent sucrose–0.01 M Tris (pH 8.3)–0.25 M NaCl) or (B) alkaline (8–22 per cent sucrose–0.25 M NaOH (pH 12.4)–0.05 M EDTA) sucrose gradients. Each gradient was prepared on a 0.5 ml. cushion of 70 per cent sucrose. Centrifugation was at 40,000 r.p.m. and 4° C for 5.5 h in the Spinco 'SW-41' rotor. Fractions were collected from the bottom of the tube and processed as in Table 1.

initiate chains, a feature that can be examined in syntheses with γ - ^{32}P -labelled deoxyribonucleotides.

All the six oncogenic RNA viruses which we have examined possess the DNA-directed polymerase, but this activity could not be detected in five non-oncogenic viruses: reo, polio, influenza, vesicular stomatitis and Newcastle disease viruses.

It is now clear that RNA oncogenic viruses contain two DNA polymerase activities: one which uses single-stranded RNA as a template and in the process generates a DNA–RNA hybrid; and a second, described here, which accepts double-stranded DNA as a template and yields a principally double-stranded product. The primary function of the latter may be to amplify the oncogenic DNA duplex once it is formed. The multiple copies so produced could markedly increase the probability of a successful integration. It is, of course, still necessary to clarify how the DNA–RNA hybrid is converted to the DNA–DNA duplex, a necessary reaction which we believe is occurring in our preparations. Is a third catalytic function required or can one of the two activities we have already identified mediate this step? The use of suitably constructed DNA–RNA structures should resolve this problem. It will be necessary to purify the relevant proteins and tackle the formidable problem of the logistics of preparing purified viruses in amounts adequate for a realistic attempt at this kind of enzymology.

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Three Dimensional Structure of the Chromatoid Body of *Entamoeba invadens*

by

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A reconstruction of the asymmetric unit of the chromatoid body helix from electron microscopic data corresponds to a particle which in many ways resembles the ribosome.

THE chromatoid bodies found in cysts of *Entamoeba invadens* are composed of ribonucleoprotein particles arranged in closely packed helices. Because of the size of the units (about 200 Å), it has been thought that the units are related to ribosomal particles, and this assumption is supported by the following evidence: most of the absorption at 260 nm occurs in the chromatoid body of the cell cross-section¹; no individual ribosome-like particles are observed in electron micrographs of sections except in close proximity to chromatoid bodies; cytochemical tests indicate that the chromatoid body contains ribonucleoprotein²; and calculation shows that the number of ribosomes expected in a cell is about equal to the number of units found in the chromatoid bodies of an average cyst³. These regular arrays of ribosome-like particles constitute a unique material for the analysis of ribonucleoprotein structure by electron microscopy, optical diffraction, and numerical image processing.

A model has been proposed⁴ for the packing of units in the chromatoid body on the basis of an optical diffraction analysis of an electron micrograph. This study concluded that the helical symmetry was described by the selection rule $l = 5n + 12m$, that is, twelve units in one helix repeat five turns long. A second study by Morgan⁵ attempted to apply a method suggested by DeRosier and Klug⁶ for the reconstruction of a three dimensional object from electron microscopic data. DeRosier and Klug⁷ raised three objections to Morgan's article and believed that analysis to be incorrect. Here, after presenting a case for choosing a somewhat different symmetry for the helix, we describe an optical and computational reconstruction of the unit of the chromatoid body helix.

Three Dimensional Reconstruction

DeRosier and Klug⁶ demonstrated the feasibility of doing three dimensional structural studies from electron micrographs in their study of the tail of bacteriophage T4. Their procedure is based on what is known to crystallographers as the projection theorem, which states that the Fourier transform of a three dimensional structure projected onto a plane is a section through the Fourier transform of the three dimensional structure. By tilting a specimen in the electron microscope one could collect sufficient data to fill Fourier space adequately. The Fourier coefficients, thus collected, could then be inversely Fourier transformed to reconstruct the specimen in three dimensions. Alternatively, they pointed out that if rotational or screw symmetry exists it may be used to lessen the number of views required to perform a three dimensional reconstruction. In carrying out a reconstruction from an electron micrograph one must consider the relationship between the distribution of absorbance on the micrograph and the distribution of stain in the specimen. To relate the distribution of stain in the object to the distribution of absorbance in the recorded image, the

amplitude contrast⁸ formalism was used. (Those micrographs which were numerically processed by computer in this analysis were all taken sufficiently close to focus so that the first interference maxima⁹ as assessed by optical diffraction patterns appeared at reciprocal spacings greater than $1/12 \text{ Å}^{-1}$. This minimized phase contrast¹⁰ at the level of resolution where we worked and thereby justified the use of the amplitude contrast formalism.)

Structural Analysis

The interpretation of micrographs of positively stained and sectioned crystalline or semi-crystalline material must circumvent a number of difficulties which are not ordinarily encountered in studies of isolated negatively contrasted particles. These involve the orientation of sections and their thickness; but sectioned materials offer the advantage of preserving dimensions¹¹ better than negatively contrasted material. Both the orientation of a

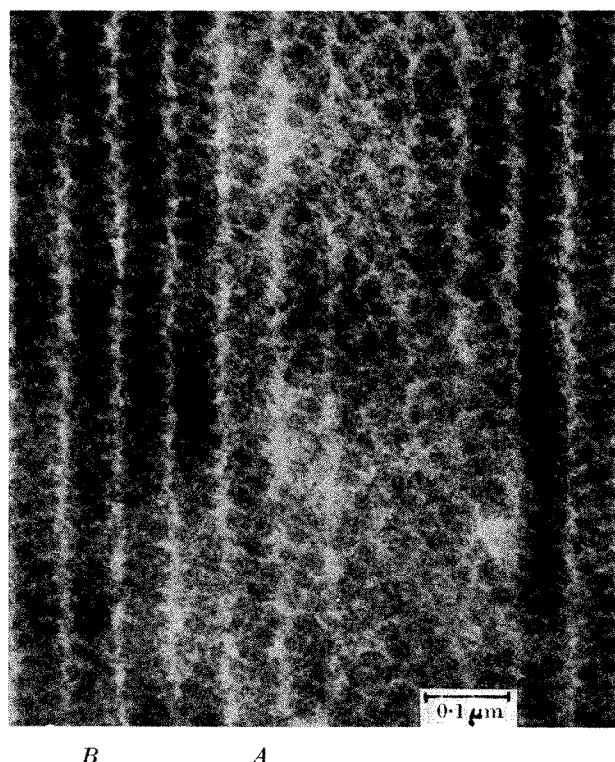


Fig. 1. Electron micrograph of a positively contrasted chromatoid body sectioned along the helical axis. "One sided" helices with intact peripheral regions are shown in region A and "two sided", truncated helices are shown in region B.

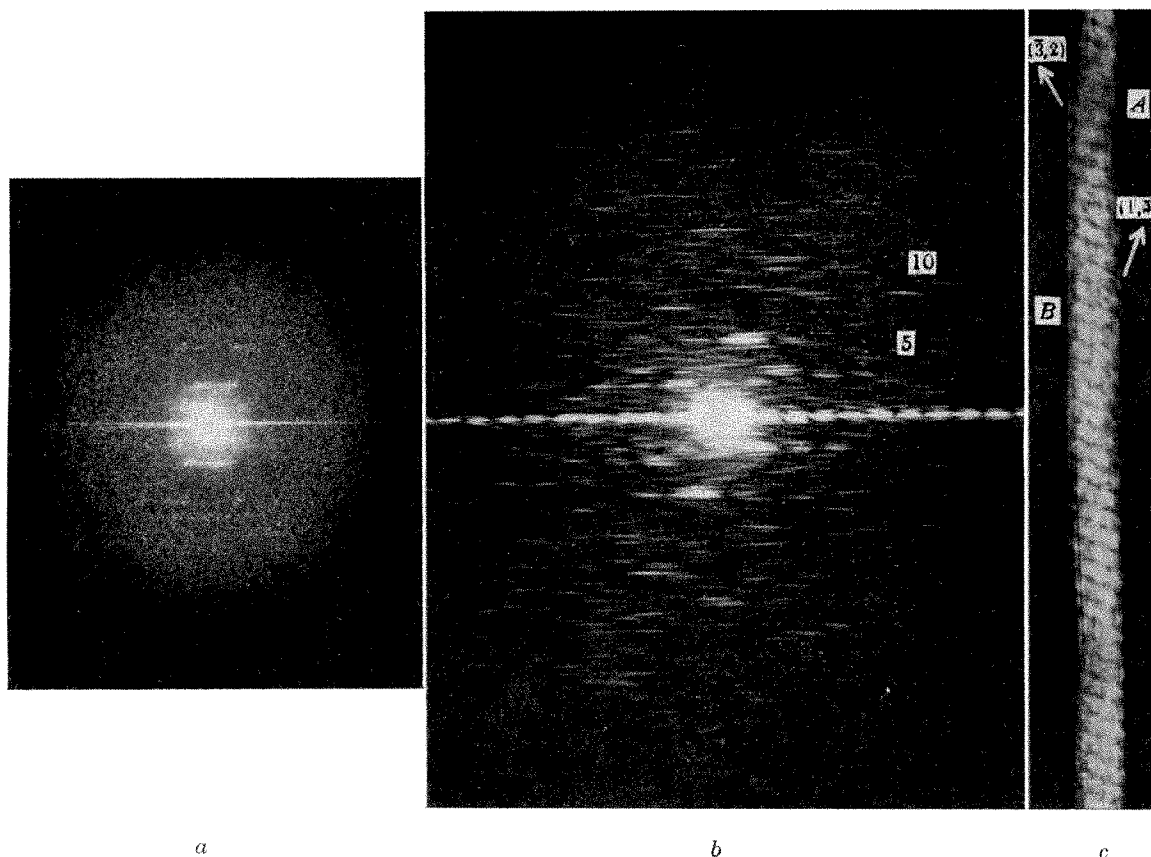


Fig. 2. *a*, The optical diffraction pattern obtained from a highly defocused image of a section of a chromatoid body (the first interference maxima is at a reciprocal spacing of about $1/70 \text{ \AA}^{-1}$), demonstrating the selective heightening of certain spatial frequencies in a defocused image. *b*, An optical diffraction pattern from a one-sided helix. The helical symmetry is described by the selection rule $l = 5n + 17m$. There are no restrictions on n . *c*, The "one-sided" diffraction pattern shown in *b* has been optically filtered to show more clearly the back half of the helix by removing noise and information arising from the front side. Most interesting are the grooves near the top of the image (region *A*) which are perpendicular to the (3, 2) direction and which give rise to the (3, 2) diffraction term. These grooves disappear near the lower part of the image (region *B*) where the outer part of the helix has been removed by sectioning. This is in agreement with predictions from the three dimensional structure. Stain is represented by light.

section and its thickness may substantially affect the electron microscope image and its optical diffraction pattern. It was found that by interpreting images of sections of the chromatoid body less than one helix diameter thick (600 Å) one could determine both orientation and section thickness quite accurately (unpublished).

A thickness of about 400 Å (as shown in Fig. 1) was found to be optimum when all factors were considered. Thicker sections provided less contrast, presumably because more lead grains overlap in the projected image. Sections less than 600 Å thick allowed an easy determination of thickness, and prevented images from being degraded by overlapping helices. With thicker sections individual helices necessarily overlapped. An analysis of the optical diffraction patterns of sections indicated that the chromatoid body was not crystalline. As a result of this lack of crystalline order (Fig. 2*a*) between helices, these overlapped images were of little value for structural analysis. In practice, the helical axes were not perfectly straight nor the sections perfectly planar so that when sections were too thick, other helices entered and left the section, causing overlapped images. These factors were balanced against the realization that using the projected image of less than one entire helix introduced errors into the three dimensional stain map. It was necessary to screen a vast number of sections of chromatoid bodies in order to select those few which were oriented very closely parallel to the helical axes and at the same time were of the appropriate thickness and quality. More than a hundred of these images were photographed and analysed by optical diffraction: those we have chosen for three dimensional

reconstruction are, in our opinion, the best thickness, compromise which is now feasible.

Individual helices were masked from images of the chromatoid body sectioned parallel to the helical axis (Fig. 1). Optical diffraction patterns (Fig. 2*b*) of these individual helices indicated that the helices could be described by the selection rules $l = 5n + 12m$, $l = 5n + 17m$, or $l = 7n + 19m$. The positions of the maxima measured from the ten most intense layer lines, indicated that the "average" radii calculated for these selection rules were 205 Å, 250 Å and 280 Å respectively. The maximum diameter of a helix was measured from the image to be $600 \text{ Å} \pm 20 \text{ Å}$. A uniform density 600 Å diameter helix has an average radius of 200 Å. This seemed to suggest a selection rule of $l = 5n + 12m$ in accordance with the analysis of an optical diffraction pattern of an entire section⁴. The Fourier transforms calculated by computer from individual helices, however, provided not only diffraction intensities but phases as well. On each layer line in the region in which contributions from only one Bessel function are present* the complex Fourier transform is either symmetrical about the meridian (when the order of the Bessel function is even) or is antisymmetrical about

* In the region of a helical transform in which there is no overlap of various orders of Bessel functions the three dimensional Fourier transform on any layer plane, L , is given by¹² $F_L(R, \psi) = G_n(R) e^{i n (\psi + \pi/2)}$ where R and ψ are the polar coordinates of a point in reciprocal space and n is the order of the Bessel function contributing to that layer plane. If the helical axis is aligned perpendicular to the optical axis of the electron microscope, the amplitudes on the right and left hand sides of the L th layer line of the transform of the electron microscope image are symmetric about the meridian while the phases differ by a factor of $e^{-i n \pi}$. That is, if n is even, the right and left hand sides of the complex transform are equal and if n is odd, the right and left hand sides are of opposite sign.

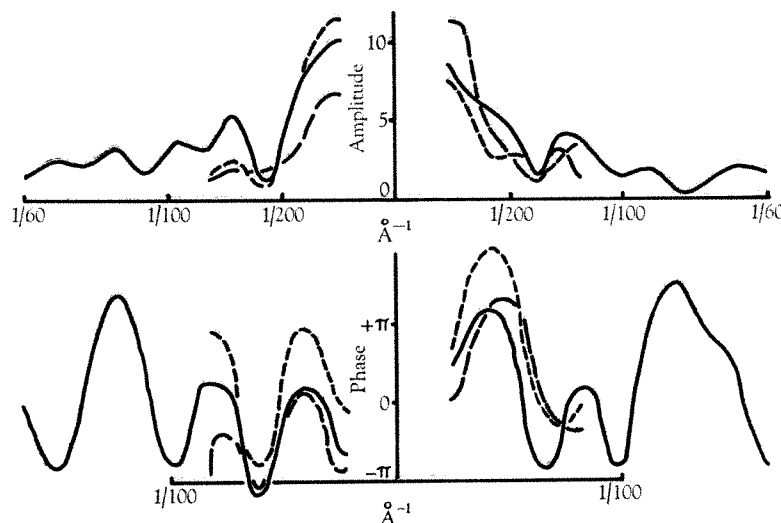


Fig. 3. Computer generated amplitude and phase along the second layer line obtained from three different "two sided" helices. In the region from $1/500 \text{ \AA}^{-1}$ to $1/170 \text{ \AA}^{-1}$, the phases on either side of the meridian differ by π in accordance with only the third order Bessel function contributing to the transform. There is no symmetrical behaviour which the phases or the amplitudes must have at reciprocal distances greater than $1/140 \text{ \AA}^{-1}$, and there is no simple relationship between the phases and amplitudes of different helices at these spacings.

the meridian (when the contributing Bessel function is of odd order). Thus from the intense fifth layer line we determined that the selection rule $l = 7n + 19m$ was invalid. The phases on the second layer line calculated from three "two sided" helices indicated that the contributing Bessel function must be of odd order (see Fig. 3)*. This, in our opinion, indicates that the helix can only be described by the selection rule $l = 5n + 17m$. Other layer lines (most notably $l = 3$ and $l = 7$) of the transforms of two sided images confirm this indexing. The helical symmetry is sufficiently high to allow us to reconstruct the asymmetric unit at a resolution of about 65 \AA from one view of a helix of sufficient length.

Summary of Procedure

The images on which the three dimensional reconstruction was based were obtained from cysts fixed for 1 h in 2 per cent glutaraldehyde (pH 7.4), washed overnight in cacodylate buffer and post-fixed in Dalton's chrome-osmium¹³, embedded in 'Epon'-'Araldite', sectioned and positively stained with lead citrate. The electron micrograph used in the three dimensional reconstruction was carefully chosen. This choice was based on criteria of orientation, contrast and order assessed by visual inspection of the image and, more important, of its optical diffraction pattern. The selected image was enlarged together with a calibrated density wedge onto fine grain film. The absorbance of the film was measured at regularly spaced points on a grid and converted, by means of intermediate emulsion calibration measurements, to a stain density by the amplitude contrast formalism⁸. In the computational process an individual helix was masked from the array of helices by an aperture. The stain density of the surrounding aperture was set equal to a constant value matching the density found on the perimeter of the helix. This procedure was found necessary in order to minimize the perturbing effect of the aperture on the calculated diffraction pattern. This masked helix was then Fourier transformed by the fast Fourier trans-

* The phases calculated from one "one sided" helix on the second layer line were symmetrical about the meridian. This is not considered significant for the phases from a "one sided" helix do not necessarily obey the symmetry relationships of a "two sided" helix. To a first approximation, the front half of a helix contributes to the Fourier transform on one side of a layer line while the back half of the helix contributes to the other half of the layer line. Thus when one examines the symmetry about the meridian along a layer line one is in effect comparing the transform of the front with the transform of the back of the helix. In a "one sided" helix one side or the other is not present and no comparisons can be made.

form algorithm of Cooley and Tukey¹⁴; it was necessary, however, to measure the stain density on a sufficiently fine grid to keep the aliasing error† small.

The formalism of Klug, Crick and Wyckoff¹² was used in carrying out the Fourier-Bessel inversion portion of the reconstruction. Because of the presence of an odd number of units per helical repeat it was possible to determine the contribution to each layer line of two Bessel terms of different order. Layer lines 0-17 inclusive were included in the reconstruction. The densities obtained from the Fourier-Bessel inversion emerged most naturally in cylindrical coordinates. These were converted to cartesian coordinates. Because one contour line adequately represented the data, 'Styrofoam' sheets were cut from templates made from the computer printout. A three dimensional model was made by gluing the 'Styrofoam' sheets together (Figs. 6a and b).

The magnification was calibrated by recording images of the 25 \AA spacing of indanthrene olive¹⁶. From images of calibrated magnification and known orientation the average of seven helical repeat distances was 1260 \AA .

Description of the Structure

A close inspection of images of the chromatoid body revealed that the lead stain was not continuously distributed throughout the specimen but existed as aggregates about 50 \AA in diameter. This was shown quite dramatically in stereo pairs of micrographs. Whether these grains were formed during the staining process or as a result of damage to the specimen in the electron beam is not known, but is being investigated.

The optical diffraction patterns of images of helices stained with lead are regular—although there is a high level of noise—to reciprocal spacings of $1/65 \text{ \AA}^{-1}$. This indicates that the lead grains are preferentially distributed on the average with a positional accuracy of about 40 \AA . There are several reasons for the relatively high noise level in positively contrasted, sectioned material compared with that in negatively contrasted images: the sectioning process frequently distorts structures, and consequently their diffraction patterns; larger lead grains exist in stain aggregates than are found in common negative contrast media; variations in section thickness can result in the splitting of layer lines.

A limiting factor in this analysis is that our sections were approximately 400 \AA thick whereas the diameter of the helix is 600 \AA . This detracts from the data and consequently from the certainty with which the peripheral regions of the helix are reconstructed. The correctness of a three dimensional structure reconstructed from a

* Cooley and Tukey used, to advantage, redundancies appearing in the Fourier transformation and greatly reduced the necessary computation time. Without their algorithm, the very large amount of data processed here would have been prohibitively expensive to transform at present day computer speeds.

† In doing numerical computations one must evaluate the continuous Fourier transform by means of sampling the continuous density at regular points of a grid, that is, a discrete Fourier transform. The difference between the continuous Fourier transform and the discrete Fourier transform is called the aliasing error¹⁵. There is in addition another error caused by the shape of the illuminating spot of the densitometer, $s(x,y)$. Taking both errors into account, one measures the absorbance $c(x,y)$ averaged over the spot shape, that is, $\int c(x+u, y+v) s(u,v) du dv$, at regularly spaced points on a grid which can be represented by a periodically spaced array of delta functions, $d(x,y)$. The Fourier transform of this is, by the convolution theorem, $[F(h,k)S^*(h,k)] \circ D(h,k)$ where $F(h,k)$ is the Fourier transform of $c(x,y)$, $S(h,k)$ is the Fourier transform of $s(x,y)$, $D(h,k)$ is the Fourier transform of $d(x,y)$ and \circ represents the convolution operation. This expression adequately approximates the continuous Fourier transform, if, first, $S(h,k)$ is essentially constant over the region of interest of $F(h,k)$ —that is, $s(x,y)$ is sufficiently localized—and second, if the peaks of $D(h,k)$ are separated by at least twice the reciprocal space distance at which $F(h,k)S^*(h,k)$ vanishes—that is, the sampling points are spaced close enough together.

sectioned helix is reflected in the degree to which the Fourier transform obeys correct phase relationships along layer lines*. Thus Fig. 3 provides an indication of the approximations involved in using sections less than one helix diameter thick. A second method of assessing this error is to compare structures reconstructed from images of partial helices sectioned through different regions. Fig. 5a and b demonstrate the type of agreement found among structures reconstructed from different helices. The helix from which Fig. 5a is derived was sectioned through the middle two-thirds of the helix while Fig. 5b is derived from a helix sectioned through the front two-thirds of the helix. Both are compared with the same overlay representing the best fit to the two structures and both agree quite well with the overlay.

The three dimensional structure of the chromatoid body helix consists of relatively discrete units (Fig. 6). The boundaries between units are in general well defined, but certain boundaries are indistinct. A principal region of contact of neighbouring units is at a radius of 100–200 Å from the helical axis. At this radius there is a region of density linking each unit with the neighbouring unit at the lower left (when the helix is viewed perpendicularly to the helical axis) and upper right. This contact accounts in a large part for the grooves perpendicular to the (1,5) direction which are so evident in Fig. 2c. The contact perpendicular to the (1,5) direction vanishes at radii greater than 200 Å and the resulting separation of neighbouring units causes the prominent groove perpendicular

* The helical selection rule for a sectioned helix is a weakened version of the selection rule for the intact helix. For an intact helix, on any layer plane only the Bessel functions given by the n,l plot for the helix can contribute to the Fourier transform of the helix. For a sectioned helix, however, density at a given radius from the helical axis is distributed in one, or two, allowed angular regions. The effect of this is that the correct n,l plot for a particular radius of a sectioned helix is now the n,l plot of the intact helix convoluted with a function which is just the Fourier transform of the function which has the value unity at angles where the helix is present and zero where the helix has been removed. The n,l plot of a sectioned helix will exist at each value of n,l permitted by the selection rule for an intact helix. These major values of n,l will be accompanied by smaller satellite values of n',l . The Fourier-Bessel inversion should reduce the contributions from the satellites. In a structure reconstructed from a helix which is about 75 per cent intact the satellite n',l peaks could be expected to contain about 25 per cent of the intensity contained in the n,l term from an intact helix. The Fourier-Bessel inversion can be expected to reduce the contributions from the satellite peaks to about 10 per cent of the total n,l intensity.

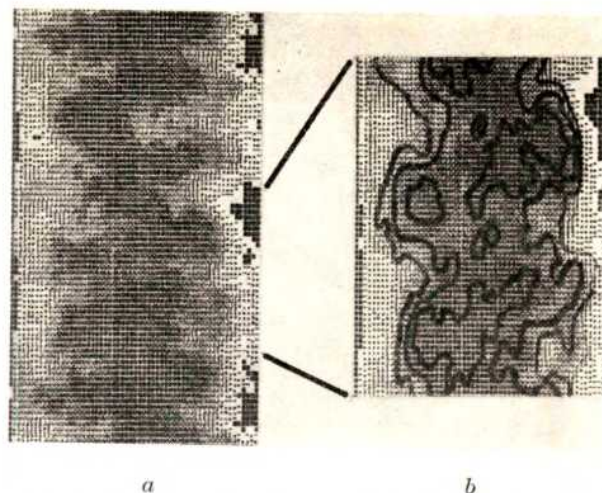


Fig. 4. *a*, A computer filtered, truncated "two sided" helix. Stain is represented by darkness. *b*, The three dimensional structure obtained from the helix in *a*, when embedded, sectioned, and projected as described in the text is shown as a contoured overlay on the filtered image. Note that shading within a contour indicates a lower density region. Also the helix has been stretched by a factor of two in the direction of the helical axis.

to the (3,2) direction. A second, although much smaller contact may exist at a radius of 150 Å, which connects each unit with its neighbours at the upper left and lower right (once again when viewed perpendicularly to the helical axis). A complex invagination which is roughly parallel to the helical axis is seen in some of the units on the left of the helix in Figs. 6 and 7. This cleft enters into the particle as deeply as 140 Å and breaks through to the edge of the particle at several places. It is a narrow feature (about 60 Å wide) but it appears consistently in the structures which were reconstructed and we think it is not an artefact. A slightly different choice of density could result in changes in the width of the cleft and in the location of its entrances and exits from the particle. Any interpretation of the structure will depend on the staining mechanism. It is thought that nucleic acids are stained

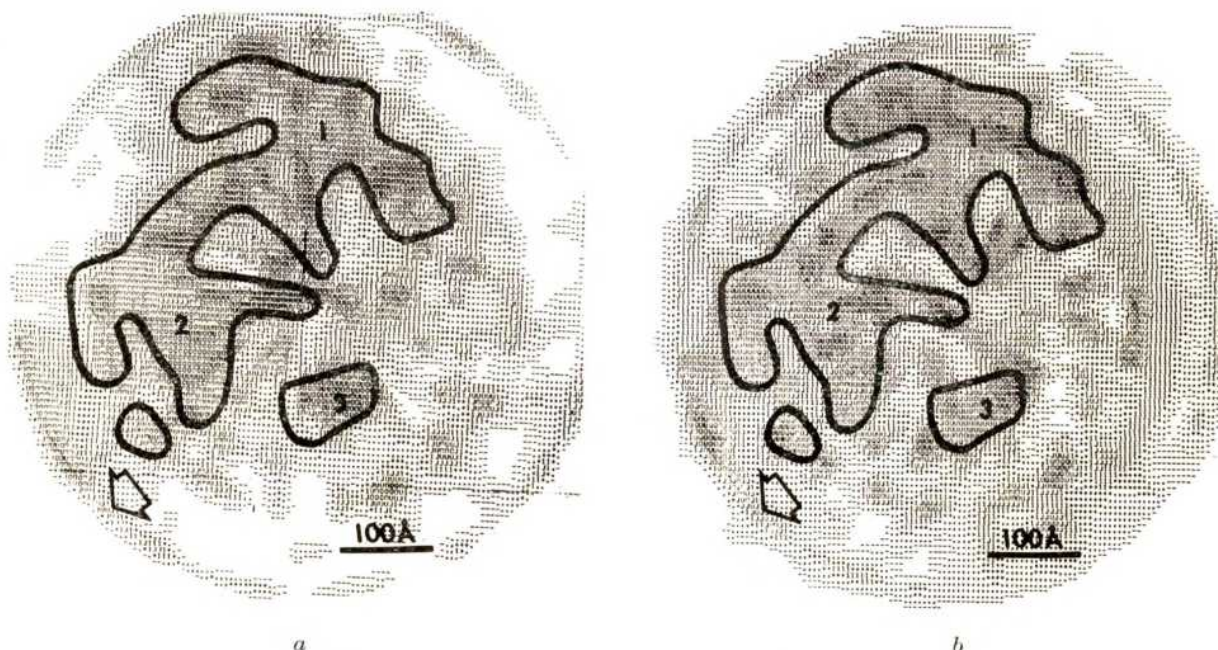


Fig. 5. A transverse sampling of the stain density map calculated from images of two differently sectioned helices. Stain is dark. *a* is the structure calculated from a helix longitudinally sectioned through the middle two-thirds of the helix and *b* is the structure calculated from a helix longitudinally sectioned through the front two-thirds of the helix. Both sections sample parts of the three different ribosomes (labelled 1, 2 and 3). The helical axis passes through the centre of the figures and is perpendicular to the plane of the page. The ripple shown by the arrow is an artefact which arises when one helix is masked from an array of interlocked helices. The ripple lies just outside the maximum radius of the helix.

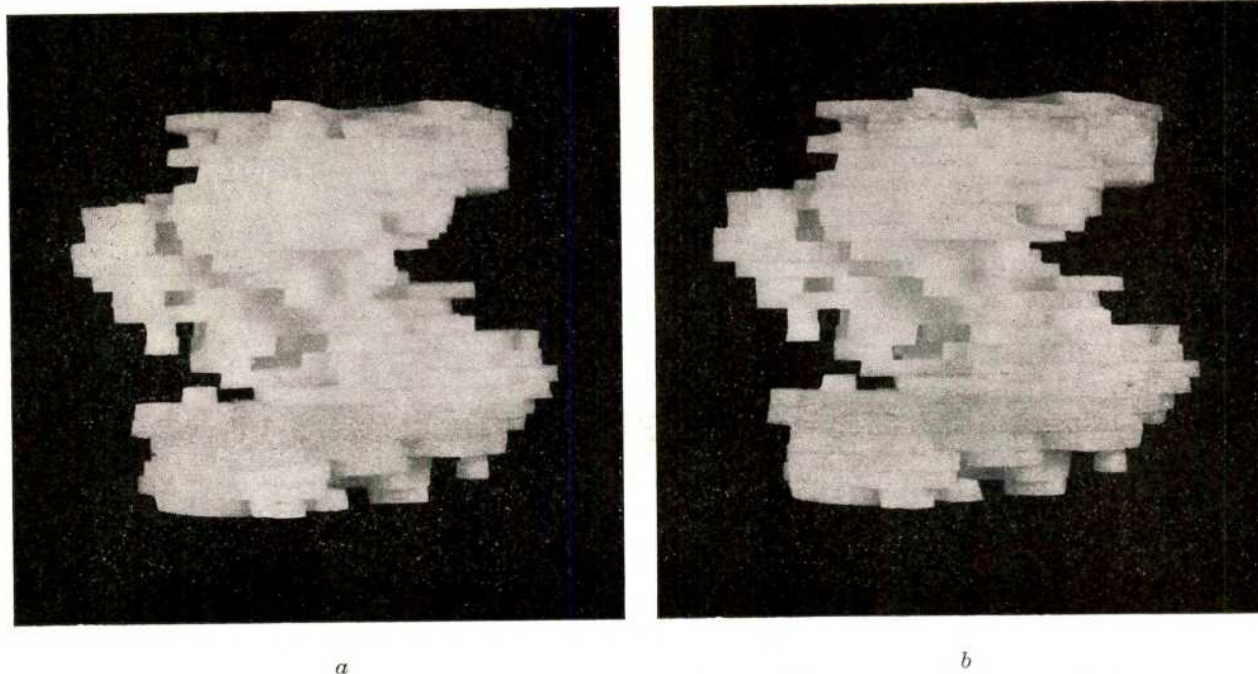


Fig. 6. A stereo pair of a 'Styrofoam' model six-seventeenths of a repeat long that represents the distribution of stain in the helix. The diameter of the helix is 600 Å.

by lead citrate more strongly than proteins¹⁷. Whether the invaginations and low density regions are holes or non-staining areas is not clear.

Both negatively contrasted images and shadowed specimens of individual helices³ suggested that the helix was right-handed, although this identification is not yet complete. An inversion of the order of the 'Styrofoam' sheets along the helical axis in Fig. 6 gives the left-handed model.

Any test of a structure rests ultimately on the comparison of the structure and the image from which the structure was obtained. The signal to noise ratio in images of the chromatoid body was so low that a direct comparison was difficult. Two different types of structural checks were carried out.

From micrographs of thin sections of known orientation (Fig. 1) it can be shown that in "one-sided" helices in which the peripheral region of the helix is intact one sees long grooves perpendicular to the (3,2) direction. Similarly, in areas in which the peripheral regions of a helix are missing (truncated helices) the long grooves mostly disappear whereas short grooves perpendicular to the (1,5) direction are intensified. This is shown more clearly by the optically filtered image in Fig. 2c. At position A in Fig. 2c the long grooves are present whereas at position B the short grooves predominate. This agrees with what we observe in the reconstructed model if the peripheral region of the helix is intact in region A and cut off in region B. Thus there is the correspondence we expect between the reconstructed model and both the optically filtered image and the unfiltered image.

A second check was made by comparing two numerically processed images. The first calculated image, shown in Fig. 4a and in a stretched version in Fig. 4b, was obtained without imposing helical symmetry by filtering the image of a two-sided helix in the direction of the helical axis to remove noise. The second calculated image, shown as an overlay in Fig. 4b, was obtained on a computer by mathematically embedding the reconstructed model helix in a matrix of uniform stain density matching the background density at the perimeter of the helix. This structure was then mathematically sectioned and projected onto a plane. The two images agree very well.

This is strong evidence for the correctness of our three dimensional structure.

Relationship of the Chromatoid Body Unit to Ribosome Structure

As already indicated the asymmetric unit of the chromatoid body helix has not been shown to be a ribosome or even a ribosomal subunit. Nevertheless, because indirect evidence leads us to suspect a relationship between the chromatoid body unit and the ribosome, we compare information concerning the unit with available information about the ribosome, fully realizing that this comparison may be premature.

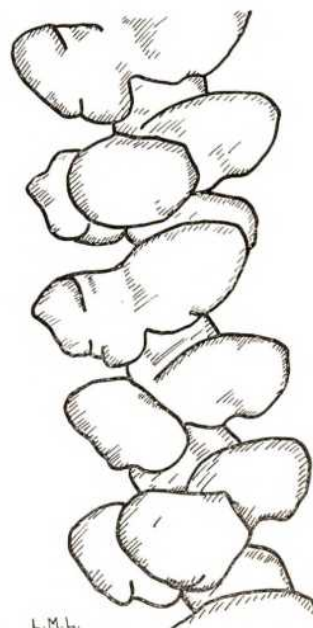


Fig. 7. A diagrammatic representation of the three dimensional structure of the chromatoid body helix. One entire helical repeat is shown.

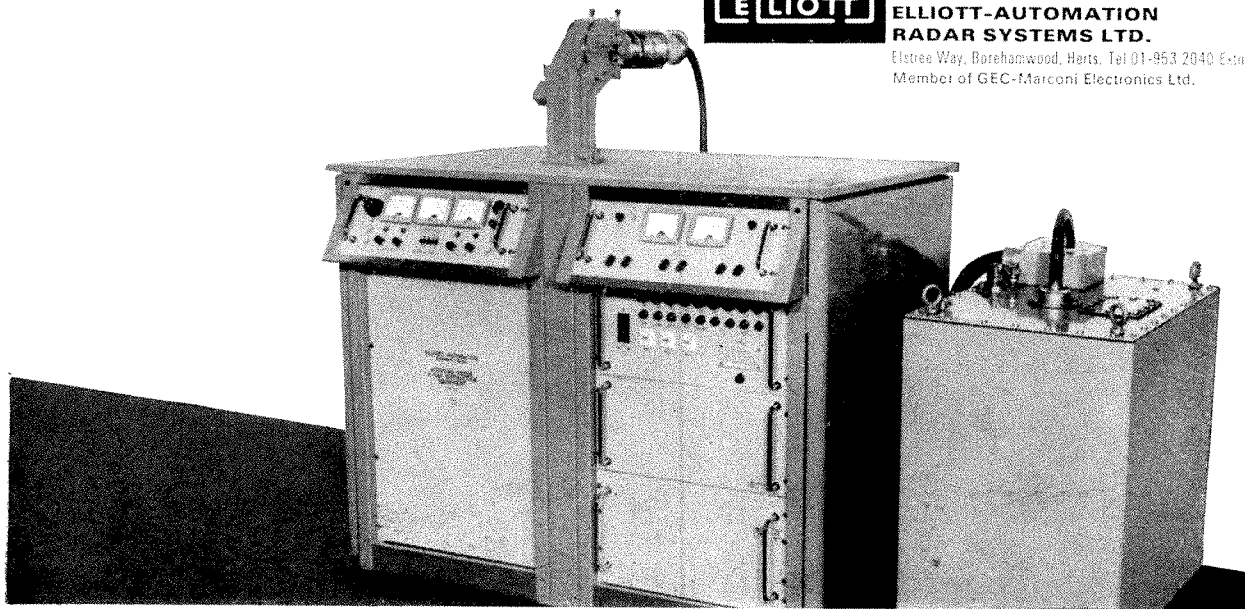
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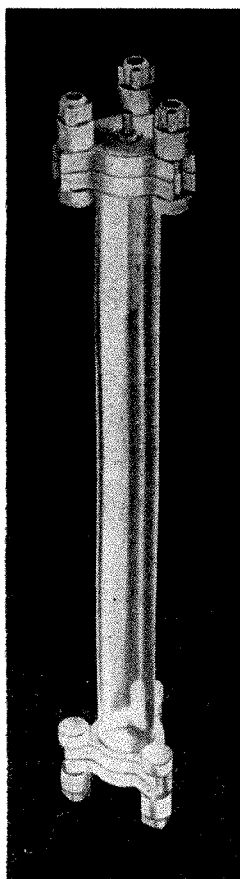


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Reported dimensions of the 70S ribosome from *Escherichia coli* vary from 140 Å to 190 Å for negatively contrasted images¹⁸⁻²⁰, from 160 Å to 210 Å for positively contrasted images^{18,19} and from 170 Å to 200 Å for metal shadowed images²¹. *Entamoeba invadens* ribosomes have, however, been found to be somewhat larger than those of *E. coli*². Also, recently small angle X-ray studies in solution²² and physical studies²³ have shown that 30S, 50S and 70S ribosomes are larger in solution than when they are observed by electron microscopy. These recent results correspond to a particle in better dimensional agreement with our structure. Sectioned, positively contrasted crystals have been shown in many studies^{11,24} to preserve dimensions whereas negatively contrasted crystals frequently exhibit smaller dimensions than those indicated in X-ray diffraction studies. We have been unable to find any part of the structure which is reminiscent of the cap-like 30S ribosomal subunit observed by Huxley and Zubay¹⁸ or Hall and Slayter²¹, although the cleft which is observed in the structure may represent a partial division between a 50S and a 30S subunit. The dimensions of one unit (roughly 360 Å × 200 Å × 190 Å) correspond reasonably well with those given by Hill, Thompson and Anderegg²² for 70S *E. coli* ribosomes (400 Å × 200 Å × 135 Å).

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Observation of Formation of Deep Water in the Mediterranean Sea, 1969

by
MEDOC GROUP*

Temperature, salinity and other measurements taken from six research vessels are discussed with regard to the formation of deep water.

Previous Indications of Deep Convection

DURING the first three months of 1969, six research vessels belonging to France, Italy, the United Kingdom and the United States were engaged in a detailed study of the winter-time formation of deep water in the northern portion of the West Mediterranean Sea. In order to describe in time and space a quickly evolving oceanic phenomenon, a fairly dense set of measurements is necessary—which accounts for the need for several ships working together. In the past there have been occasional winter-time hydrographic sections which have shown evidence of deep convection. For example, the Mediterranean Atlas¹ shows a section (number 13) made by RV Atlantis in February 1961 with a narrow region in which the vertical stability appears to be much reduced;

but of course with a single section it is impossible to know whether the section actually passes through the centre of the phenomenon, and whether the process is beginning or ending. As another example, during January–March 1963 the French naval vessel Originy made repeated observations on the 6° E meridian south of Toulon. During that winter—a particularly cold, but not very windy one—vertical homogenization of density at some stations occurred all the way to the bottom (2,500 m); but at nearby (5 miles) stations the density stratification was not markedly disturbed². Comparison of hydrographic sections made at weekly intervals showed that this phenomenon of small-scale patchy convection appeared in a week's time. After a full month of severely cold weather, the homogeneous water was encountered in more extensive portions of the section—up to 40 miles. The centre of the homogeneous region appeared to be about 40 miles south of Toulon.

These various indications of the presence of a very active vertically convective region south of Toulon provided the motivation for the joint work described here: a multiple ship operation in January–March 1969, which yielded an unusually complete picture of the process of formation and sinking of deep water in wintertime. It is the most completely documented example of the phenomenon of deep convection that we have anywhere in the ocean.

In any season the area is characterized by a large

MEDOC GROUP*

Country	Research vessel	Institute	Authors
France	NO Jean Charcot	Laboratoire d'Océanographie Physique, Paris	H. Lacombe
	BO Originy	Bureau d'Etudes Océanographiques, Toulon	P. Tchernaia M. Ribet
Italy	RV Bannock	Oceanoboe, San Terenzo	R. Frassetto
	HMS Hydra	MOD (Navy), Hydrographic Department, London	J. C. Swallow
United Kingdom	RRS Discovery	National Institute of Oceanography, Wormley	J. C. Swallow
USA	RV Atlantis II	Woods Hole Oceanographic Institution	A. R. Miller
		Massachusetts Institute of Technology, Cambridge.	H. Stommel

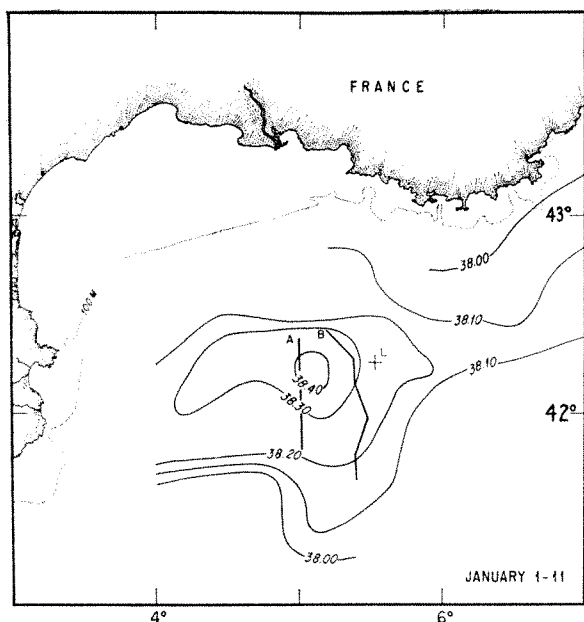


Fig. 1. Hydra survey of surface salinity, January 1-11, 1969. The line A shows position of section in Fig. 2. The line B shows position of section in Fig. 3. "L" is position of the Bouée-Laboratoire.

cyclonic gyre and the stability of the density stratification is lower in the centre than on the rim. In winter we can distinguish three phases. The first is the *preconditioning phase*, when every year, winter surface cooling occurs in the area and reduces the stability of the surface layer everywhere, leaving very little reserve buoyancy in the centre of the gyre. Vertical mixing takes place in the surface layer, the system remains a distinctly three layered one: the fresh cold surface layer, the salty warm intermediate layer, and the medium salinity, cold deep layer. The maximum near-surface density in the centre of the gyre reaches or exceeds $\sigma_t = 29.00$. In 1969 the onset of the NW to N winds marked the beginning of a second phase: the *violent mixing phase*, in which a rather narrow region of deeply penetrative convection occurs in the centre of the gyre, but other regions of the gyre are only slightly perturbed. When the stormy period ceases, the mixed water sinks rather quickly and spreads out horizontally: the *sinking and spreading phase*.

The Preconditioning Phase

During January 1-11, the British hydrographic survey vessel HMS Hydra scouted the area in the northwestern Mediterranean shown in Fig. 1, and found at the surface a region of low temperature, high salinity, and high density extending in a long strip from about 50 miles south of the Gulf of Lions into the Ligurian Sea. In the vertical the water was everywhere gravitationally stable. Although there were two storms during January, when the Woods Hole vessel Atlantis II resurveyed the region of highest surface density surrounding 42°N 5°E during January 24-28, it found that no marked change had occurred. The vertical section of salinity shown in Fig. 2 was taken by Atlantis II (the location of the section is shown by line A in Fig. 1) on January 31-February 1, and exhibits the well known three superposed layers of water generally present in the Mediterranean year-round. The intensity of these contrasts is diminished toward the centre of the section, underneath the surface patch of high salinity, but there is vertical structure everywhere.

The Violent Mixing Phase

During the evening of February 3 the NW to N wind began to blow strongly, and persisted with occasional lapses for most of February (Fig. 5b). During February 4

and 5, for example, it blew with speeds of approximately 35 knots and wet-bulb air temperature as low as 3°C . Using conventional formulae for calculating air-sea exchange processes, and regular surface meteorological observations obtained at the Bouée-Laboratoire (point L in Fig. 1) the evaporation seems to have been as much as 2 cm/day. A vertical section of salinity and temperature (Fig. 3) taken (on line B in Fig. 1) after the storm had been blowing 6 days (February 10-11) shows that strong vertical mixing did indeed occur, but not uniformly over the entire section. The central vertically mixing region is 1,400 m deep with essentially uniform salinity and temperature³.

It appears from comparison of vertical profiles that the properties of this mixed layer can be accounted for by simple vertical mixing of properties previously present brought about by strong surface cooling, and some admixture of shallow surface waters blown in from the north. Why the central mixed region is so narrow (some 25 miles) is somewhat obscure. Observations by various cooperating ships indicate that after the storm ending on February 17 the mixing had penetrated nearly to the bottom and the mixing region extended in a narrow strip with its axis oriented parallel to the coast.

The geographical extent of the mixing region, as a function of time, is well shown by the area at the surface in which salinity exceeds 38.42 parts per thousand, this high salinity being a sensitive indication of the presence of intermediate water mixed up from below. Fig. 4 shows areas of the surface where salinity exceeds 38.42 parts per thousand at four different times. The area outlined by the quadrilateral (1) was fairly densely surveyed by Hydra in the period January 1-11; only at two isolated points (marked by +) was there such high salinity measured. At this stage there was essentially no mixed water present. The triangular and rectangular areas (2) were surveyed during February 3-12 by Charcot, Atlantis II, Discovery, Origny and Bannock. A region of salinity in excess of 38.42 parts per thousand was found as shown in the figure, centred at 42°N 5°E . The later survey of February 15-28 by Charcot, Atlantis II, Discovery and Bannock in the very irregular area (3) shows this same area, but also another high salinity area in the Ligurian Sea. The final survey by Charcot, during March 18-31 in area 4 (no

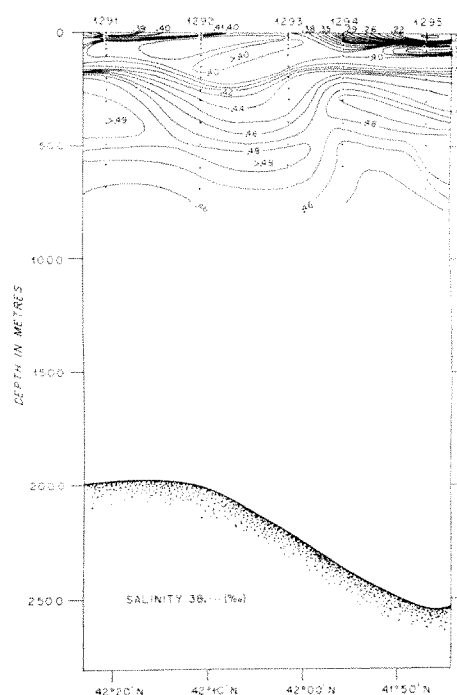


Fig. 2. Salinity on Atlantis II section A (January 31, 1969).

observations in the Ligurian Sea), shows that the high salinity is now completely gone; at one point—marked by the crescent—the surface salinity actually reached 38.42 parts per thousand, but did not exceed it. The expanding and contracting of these areas in time in the region south of Marseilles actually gives a cinematographic representation of the presence of the deep mixed layer where it reaches the surface. On the other hand, the region indicated in the Ligurian Sea exhibits higher temperatures and lower densities; the mixed layer there was not observed to exceed 500 m depth, and this did not appear to be a source of deep water.

At this time, a variety of water types was present in the strip, west of 6° E, ranging from $\theta = 12.78^\circ \text{C}$, $S = 38.420$

parts per thousand to $\theta = 12.90^\circ \text{C}$, $S = 38.450$ parts per thousand. The water type of higher potential temperature had a dissolved oxygen concentration somewhat exceeding 4.55 ml./l. This high oxygen serves to differentiate that mixed water type from water elsewhere in the same range of temperature and salinity (but lower oxygen), so that we can use it as a "tag" to show the remarkable process of sinking, spreading and breaking up of the mixed water during the last portion of February and late March.

The Sinking and Spreading Phase

Repeated profiles were run along 6° E by Atlantis II and Charcot to document the time history of the phenomenon. Fig. 5a is a time against latitude plane showing the

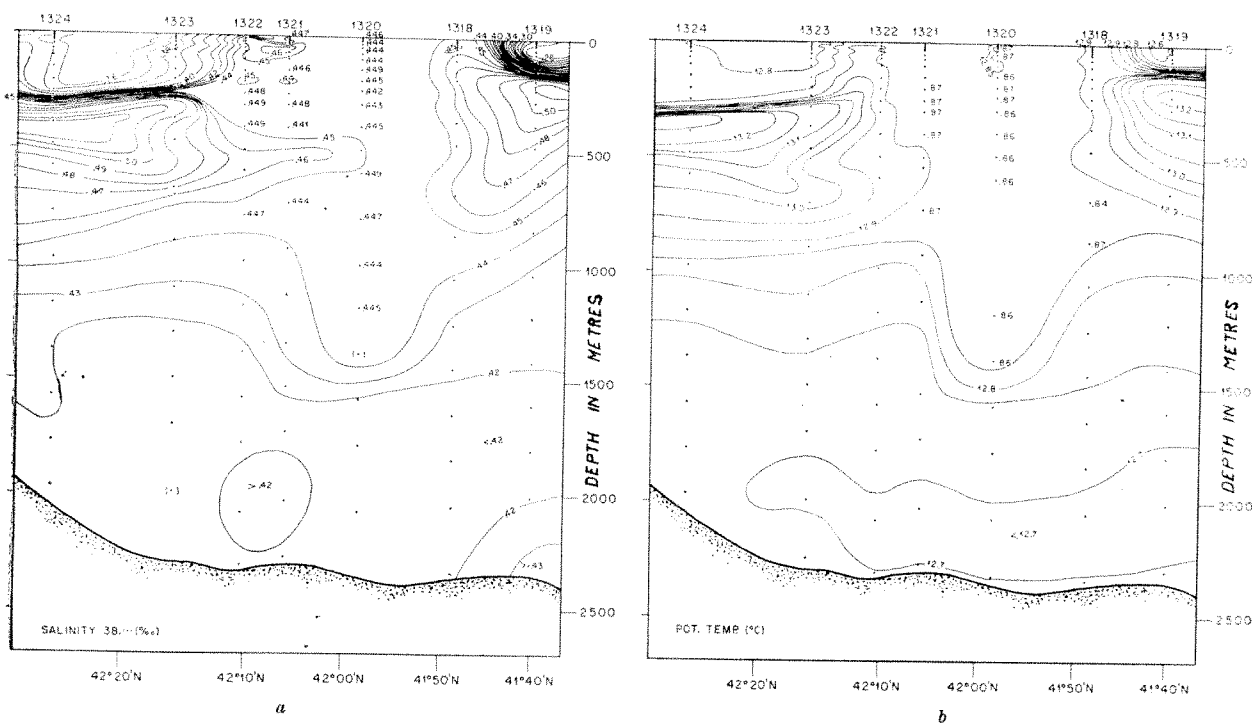


Fig. 3. *a*, Salinity on Atlantis II section B (February 10-11, 1969); *b*, potential temperature on same section.

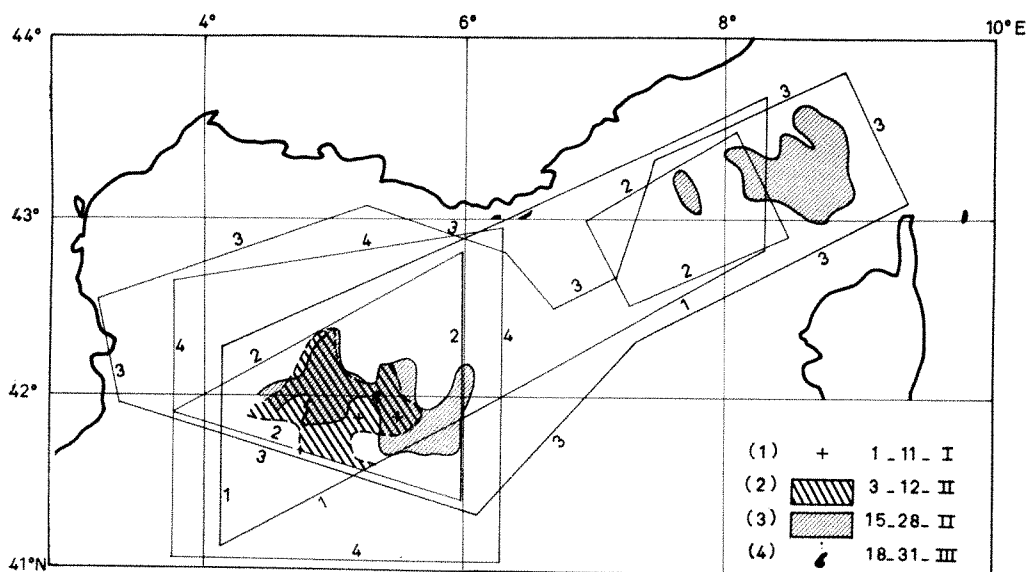


Fig. 4. Regions where surface salinity exceeds 38.42 parts per thousand during four different surveys. Total area of each survey also shown by numbered polygons.

positions of deep stations by the dots. Where there are no figures, there was no "tagged water" present. Elsewhere the figures indicate the depths (in hundreds of metres) bounding the "tagged water". Large amounts of "tagged water" are first observed on the second Charcot section (the first made after the storm): it extends from the surface to depths of up to 1,450 m, in a region about 30 miles wide, centred near 42° N. Subsequent profiles indicate sinking of the top boundary, but a fairly steady bottom boundary, and spreading north and south at about 2 miles/day. Fig. 6 shows the quite irregular geographical distribution of the "tagged water" during the Charcot survey of March 18–31, which suggests that the striated structure shown in Fig. 5a is not representative of the spatial structure of the advanced state of decay of the sinking process, but that many large fragments form.

The water type of lower potential temperature (see above) formed near 42° N, 5° E is probably very slightly more dense than the warmer variety and cannot be traced by its dissolved oxygen content. Below 1,000 m, however, its potential temperature and salinity were characteristically higher than those of the old deep water. An examination of vertical temperature profiles obtained on an expanded scale from the TSD probe used on board the Discovery shows that the spreading of the new deep water—as identified by anomalously high temperature—followed a pattern broadly similar to that of the new deep water at slightly less depth "tagged" by oxygen. Some of it evidently moved southwards from the region of violent vertical mixing at rates of at least 2 miles per day. In one place the vertical profile of potential temperature indicates that a layer of old deep water 700 m thick has been lifted upwards from 1,800 m to 1,000 m by an intrusion of new deep water.

The density of the violently mixed layer approaches

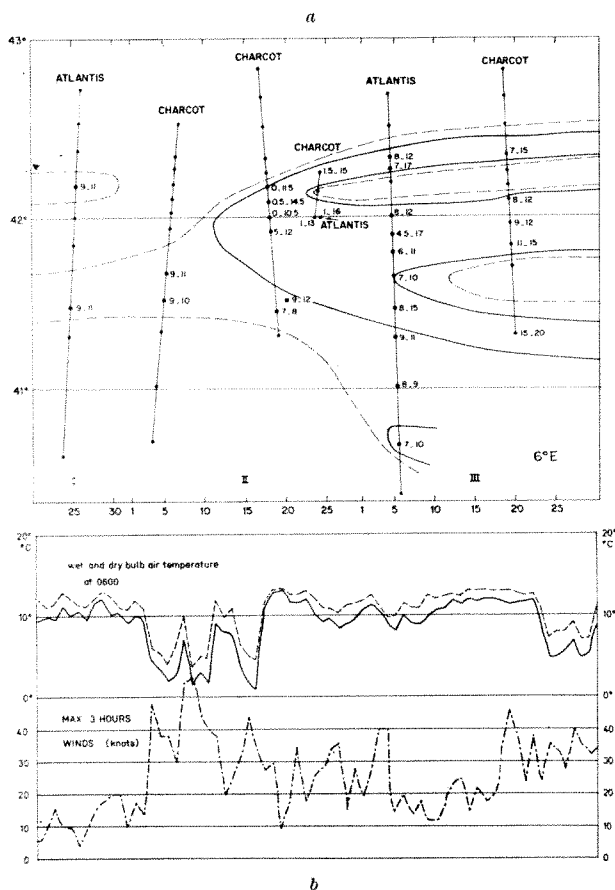


Fig. 5. a, Time against latitude diagram showing evolution of mixed water that is tagged by high oxygen. b, Simultaneous meteorological observations at the Bouée-Laboratoire.

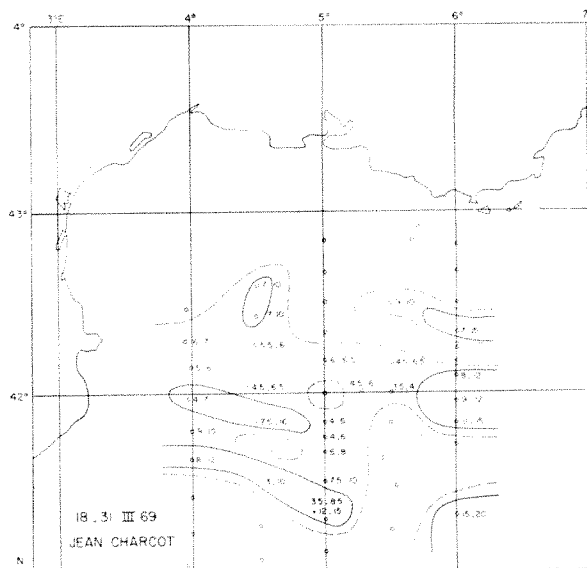


Fig. 6. Charcot survey of March 18–31, 1969, showing geographical dispersal of tagged water.

that of the bottom water very closely, to within the accuracy of determination of density: ± 5 parts per million. The deepest penetration observed was at Discovery station 6844 on March 8, when it reached a depth of 2,430 m. Even in this case the bottom-most water remained distinguishable by its slightly lower temperature and salinity.

The Velocity Field

The RRS Discovery tracked twenty neutrally buoyant floats in the region near 42° N, 5° E, and set ten moorings each with three or four current meters. Three of the moorings were in place during the storms of February 12–16. Horizontal speeds were only slightly higher during the storm, and the spectrum of kinetic energy density shows a fairly uniform increase by a factor of about 2 at periods from 10 minutes to 10 hours. At longer periods, the motion appeared weak and disorganized until February 16, after the storm, when a definite eastward flow appeared. Floats revealed a cyclonic shear across the strip of dense mixed water, with eastward flow along its southern edge and westward to the north. By February 25 the mixed water had almost disappeared from the surface, and the predominant horizontal motions were similar to what might be expected of a field of eddies with diameter about 10 miles, tangential speeds decreasing downwards from about 15 cm/s at 10 m to 7 cm/s at 1,500 m, axes vertical, migrating irregularly at speeds of approximately 3 cm/s.

An isolated attempt by Atlantis II to measure vertical component of velocity in the mixed layer, using a neutrally buoyant float, rotated about a vertical axis by vanes, indicated at one time an upward vertical displacement of 800 m in 14 hours⁴.

There is an approximate equality between the amount of dense new water formed in the middle of the strip, and that seen later during March at depths around 2,000 m; it appears to have been enough to feed the entire Mediterranean outflow at Gibraltar for about 6 weeks.

This brief report is meant to convey the main results of the multiple ship operation; the detailed documentation will, we hope, appear in later papers written individually.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Geochemical Evidence for Ocean Floor Spreading in South Atlantic Ocean

The basal sediment layer on the flanks of spreading ridges should be enriched in iron and relatively poor in aluminium if present concepts of ocean floor spreading are correct^{1,2}. Recently published evidence from the third leg of the JOIDES expedition corroborates this hypothesis.

During this part of the expedition several stations were occupied at approximately 30° S and cores were taken at stations 14, 15, 16, 19, 20 and 21, between the Mid-Atlantic Ridge and South America. Rates of deposition, chemical compositions, water contents and densities were determined on the sediments from these cores³.

The reported results³ show that the ratio $Al/(Al+Fe)$, which has been useful in the delineation of anomalous sediments on spreading ridges^{1,2}, shows a distinct decrease with depth (see Fig. 1). The most intensively studied cores came from station 19; the results³ can be recalculated to show the accumulation rates for Al and Fe (see Fig. 2). The upper 100 m show normal pelagic accumulation rates, but in the lowest 40 m the accumulation rates for iron are anomalously high, resembling rates observed in recent sediments on the crest of the East Pacific Rise^{4,5}.

The evidence in Figs 1 and 2 strongly suggest that the formation of iron-rich crest sediments has taken place more or less uninterrupted since the middle Eocene, which is the age for the basal layer at station 19. The variations in thickness of the iron-rich basal layer may have resulted from the varying intensity in ocean floor spreading and volcanism or from intrusive basalt sills which would hide the true basement. Some of the undulations of the basement (see Fig. 1) are certainly also related to the fact that some stations (Nos. 20 and 21) are occupied on the Rio Grande Rise.

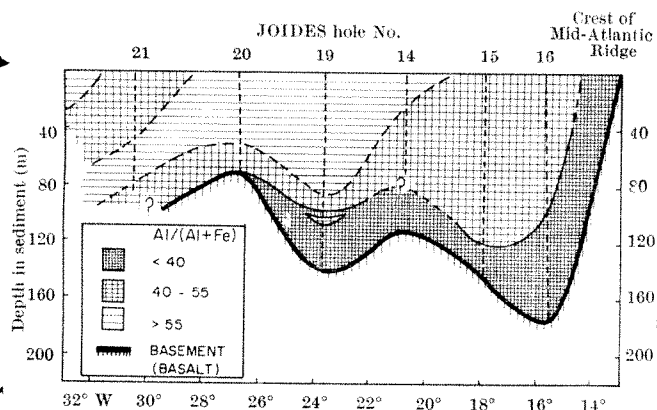


Fig. 1. Cross sections of sediments at about 30° S in south Atlantic Ocean. The number of the station is given above the figure and corresponding position (°W of Greenwich) below (for details see ref. 3). The ratio $Al/(Al+Fe) = 0.40$, is used to separate normal and iron-rich basal sediments. The lowermost part of the sediments locally have $Al/(Al+Fe)$ values less than 0.20.

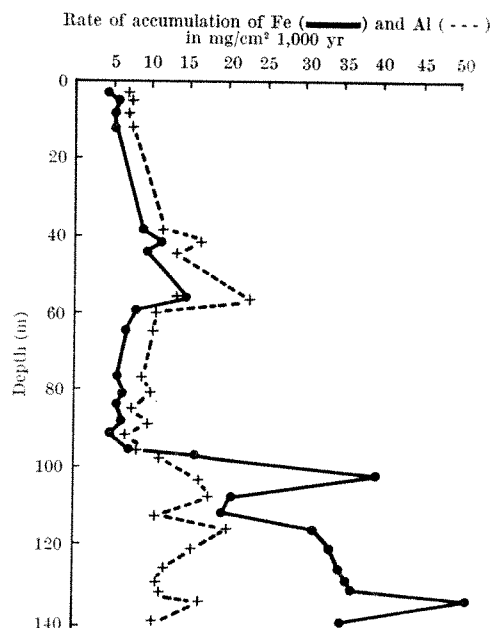


Fig. 2. Accumulation rates of iron and aluminium in sediments from station 19.

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Temperature Gradient above the Deep-Sea Floor

At sufficient ocean depths (several km) there is an increase of temperature with depth corresponding to the adiabatic lapse rate $\Gamma \approx 10^{-6} ^\circ\text{C}/\text{cm}$. Toward the bottom, the gradient becomes increasingly superadiabatic, because the water is heated from below by a geothermal heat flux H . Because H is small ($\sim 10^{-6}$ calories $\text{cm}^{-2} \text{ s}^{-1}$), only a slight superadiabatic effect is expected: $|dT/dz| < 2\Gamma$ at elevation $z > 1$ m. Measurements of hyperadiabatic gradients ($|dT/dz| = 10-1,000 \Gamma$) several metres, or even tens of metres, above the bottom have been reported¹⁻⁴, though it seems inconceivable that a strongly unstable layer several metres thick can persist. The experiment described here indicates that $|dT/dz| = 1.3 \Gamma$ at $z \sim 1$ m.

Critical Rayleigh number and Reynolds number considerations indicate that heat and momentum are transferred by molecular processes in a layer of thickness $h = 1$ to 4 cm just above the (smooth) sea floor⁵. Above this conductive layer there is convection. Townsend⁶ describes three regimes which he terms "forced", "mixed", "natural" (Fig. 1). For various elevations and typical shear stresses in the deep sea, Table 1 shows the predicted convection type and temperature gradient relative to the adiabatic gradient $-\Gamma = -1.2 \times 10^{-6} ^\circ\text{C}/\text{cm}$. The predicted slightly

superadiabatic temperature gradient contrasts with the reported measurements of hyperadiabatic gradients—for example, -15Γ to -65Γ in the layer $z=100-300$ cm (ref. 4). My initial measurements in this layer likewise indicated hyperadiabatic gradients ($\sim -100\Gamma$), but these were found to be caused by sensor self-heating and are thus spurious. Design changes have eliminated this “anemometer effect”⁷, and the ambient gradient has been measured.

Five Hewlett-Packard quartz-crystal temperature sensors were mounted on a frame and connected to a Snodgrass recording capsule⁸. With the frame standing on the sea floor, three of the sensors (I, II, III) were fixed at elevations 150 cm, 150 cm and 10 cm; two sensors (IV, V) were on a sliding vertical boom initially at elevations 290 cm and 150 cm, and after the boom was dropped, at 150 cm and 10 cm (see Fig. 2). The horizontal separation of the sensors was 10 cm. To soften the “boom-drop”, drag flaps were deployed at the boom top and a sponge-faced plate was attached to the bottom.

This arrangement allowed an *in situ* relative calibration of the sensors. The recorded time series were first converted to degrees Centigrade using laboratory calibrations. Then averages were computed for 1 h just before and another just after the boom-drop. It was assumed that two sensors at the same level should give the same average reading, any difference being calibration error which can be removed by a uniform additive correction to one or other series. In this way, all the other sensors were calibrated *in situ* relative to one upper fixed sensor (I) chosen as a “standard”. Calibration corrections from before and after the boom-drop were possible for the other upper fixed sensor (II). These corrections differ by only 1 part in 10^3 , implying that 1 h is, (i) an averaging time long enough to eliminate the random effects of small-scale turbulence, and (ii) an interval short enough that sensor drifts are unimportant.

The apparatus was dropped at $31^\circ 02' N$, $119^\circ 48' W$ (depth 3.7 km). The sampling interval was 1 min and the least-count resolution $1.7 \times 10^{-5}^\circ C$. After settling on bottom, the drag flaps were deployed. A day later, the boom was dropped. After another day, the apparatus was recalled to the surface for recovery.

A similar apparatus with a camera was placed within 2 km of this location on a previous occasion⁹. The topo-

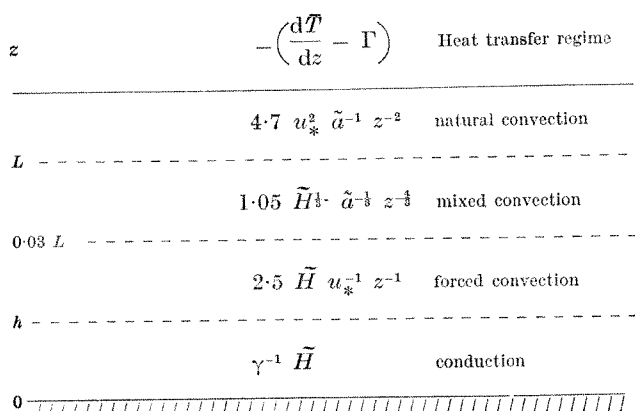


Fig. 1. Theoretical relations for the mean potential temperature gradient within the benthic boundary layer. $H=H/c_p q$; $\tilde{\alpha}=ga$; $L=2.5 u_*^2/\tilde{H} \tilde{\alpha}$. (There is doubt that the relation given for “natural” convection is correct.)

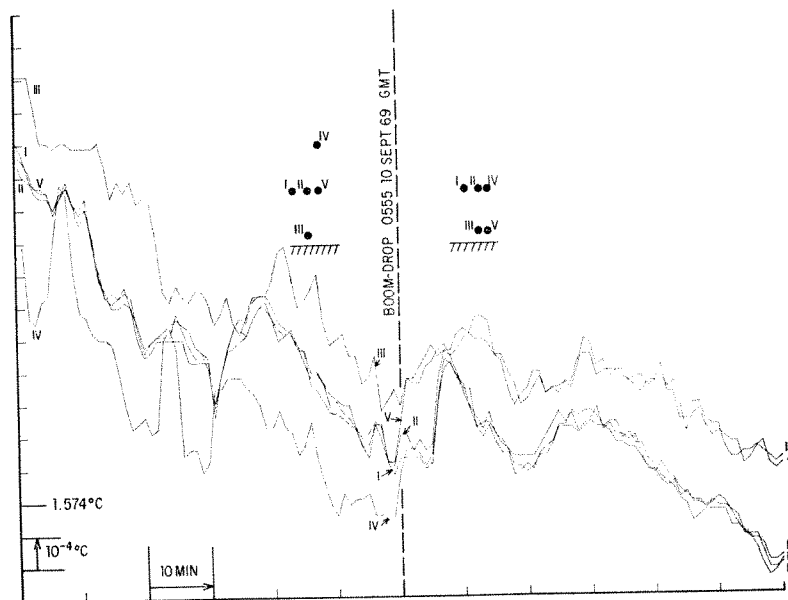


Fig. 2. Temperatures recorded near the deep-sea floor; at elevations $z=10, 150, 290$ cm until boom-drop, thereafter at the two lower levels only.

graphy in the area appears to be uniform, so it is reasonable to suppose that the 3 cm sediment penetration and the smooth bottom, indicated in those photographs, also apply to this experiment. Simultaneous with this drop, and within 0.5 km of its location, a current direction time series was recorded with a second Snodgrass capsule.

Fig. 2 shows the corrected temperature records for the 2 h period centred on the boom drop. The current direction record from the adjacent capsule indicates that during this period the flow past the sensors was directed towards the instrument frame so the influence of the frame on the temperature records should have been slight. It is immediately apparent from the figure that temperature decreases with elevation, as expected. The mean gradients, implied by the corrected 1 h averages from just before and after the boom-drop, are given in Table 2. These values indicate $|\overline{dT/dz}|=1.3\Gamma$ in the layer $z=10-290$ cm. This agrees well with the theoretical predictions of Table 1; however, there are two surprises.

The theory predicts that temperature difference between 10 cm and 150 cm should be about four times that between 150 cm and 290 cm. But the measured differences are approximately the same. (It may be that the boom-drop, though gentle, disturbed the sensors slightly. A shock-induced change in the sensor crystal frequency of just 1 part in 10^8 could account for this result.)

In Fig. 2, the lower temperature difference vanishes briefly four times, and the upper difference (recorded only until boom-drop) vanishes once. At a later time (not shown) during a tidal reversal of current direction, the record shows isothermal conditions persisting for 3 h.

Table 1. THEORETICAL CONVECTION TYPE AND TEMPERATURE GRADIENT RELATIVE TO THE ADIABATIC GRADIENT $-\Gamma = -1.2 \times 10^{-6}^\circ C/cm$

z (cm)	0.02	0.04	u^* (cm/s)	0.1	0.2
1,000	Natural	Natural	Mixed	Mixed	Forced
100	1.01	1.03	1.02	1.02	1.02
10	Natural	Mixed	Mixed	Forced	Forced
	1.9	1.4	1.4	1.3	1.2
	Mixed	Forced	Forced	Forced	Forced
	10.4	9.1	5.6	4.2	2.6

Table 2. MEASURED AVERAGE TEMPERATURE GRADIENTS DURING HOUR BEFORE AND HOUR AFTER BOOM-DROP

z (cm)	Before	After
290		
150	-1.6×10^{-6}	
10	-1.5×10^{-6}	-1.6×10^{-6}

Does the metal frame act as a thermal "short-circuit" to the water flowing by? Rough calculations indicate that conductance through the vertical frame members and their associated boundary layers is insufficient. But the flow past these members has a Reynolds number of only 10^2 to 10^3 , so it may be a quasi-steady separated flow with trapped eddies enclosing the sensors in the wake¹⁰. Thermal "shorting" of these eddies might be achieved and an isothermal profile would then be indicated. If this is correct, then the brief isothermal incidents in Fig. 2 presumably occurred during momentary current reversals.

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Planetary-type Waves in the South West Indian Ocean

SOUTH of the latitude of the Cape of Good Hope, the Agulhas Current Stream (or at least most of it) turns back into the Indian Ocean and forms a zonal current called the Agulhas Return Current, whose mean axis of flow is approximately 38° – 39° S. Near the start of its eastward flow, the Agulhas Return Current has been shown to undergo sharp changes of direction (Fig. 1a). These deflections are apparent in the analysis made by Dietrich¹ and Le Pichon². The latter shows them occurring down to the depth of the salinity maximum and draws attention to the important influence of bottom topography. They are also very evident in the study made by Darbyshire³ of data from the cruises of SAS Natal and RS Africana II, occurring in three out of the four seasons examined. The deflections all occur near a prominent topographical feature, the Agulhas Plateau, and their recurrence suggests that they are a quasi-stationary feature and possibly planetary waves caused by the rapid changes in water depth. An investigation into this possibility has been made by comparing the flow patterns deduced from hydrological data with those predicted by theory.

The theoretical model we used was developed by Porter and Rattray⁴ for zonal flow over meridionally oriented topographical features, of a frictionless, steady (and initially uniform) barotropic current. Using the beta-plane approximation, they found a solution in

terms of the transport stream function and based on the conservation of potential vorticity. The obvious departure of this model from the baroclinic, non-uniform, time dependent, real ocean was emphasized by the authors.

The Agulhas Return Current seems to approximate to zonal flow over some meridionally oriented topography. It has, however, strongly baroclinic features so that close agreement between theory and observation is not to be expected.

We used Porter and Rattray's equations (8) and (11) for the calculations, together with the following data: width of the Agulhas Plateau = 260 km; initial depth, H = 5,000 m; depth over the Plateau = 3,000 m; depth immediately down stream of the Plateau = 4,200 m (for other depths and distances see Fig. 1c); value for beta in beta-plane approximation = $1.8 \times 10^{-8} \text{ km}^{-1} \text{ s}^{-1}$; initial velocity to the east, U , (assumed to be about the maximum barotropic velocity) = 10 cm/s.

Bathymetric data was taken from charts prepared by the South African Hydrographic Office and by Simpson and Forder⁵, and an approximate profile drawn for depth along 39° S. (Fig. 1c). The simplified profile from which the computational data were obtained is indicated by a broken line.

The predicted flow pattern is shown in Fig. 1b, where the normalized stream function — UHy/ψ is plotted against the zonal distance in degrees longitude. A chart of the surface dynamic isobars with reference to the 1500 db surface was drawn for intervals of 1 dynamic decimetre (Fig. 1a). Data from the following cruises were used: SAS Natal, 1962⁶; RS Africana II, 1962⁷ and 1961⁷; and RV Anton Bruun, 1964⁸. The first two cruises were almost synoptic and formed part of Darbyshire's³ study. All stations were occupied in the month of July or within a few days of it. The choice of the reference level was dictated by the limiting depth of key stations rather than by any conviction that it was a level of no motion—which it almost certainly is not. Where, because of the sparseness of stations, the contouring became especially subjective, the contours have been marked with broken lines. Light shading has been used to show the 4,000 m, and heavier

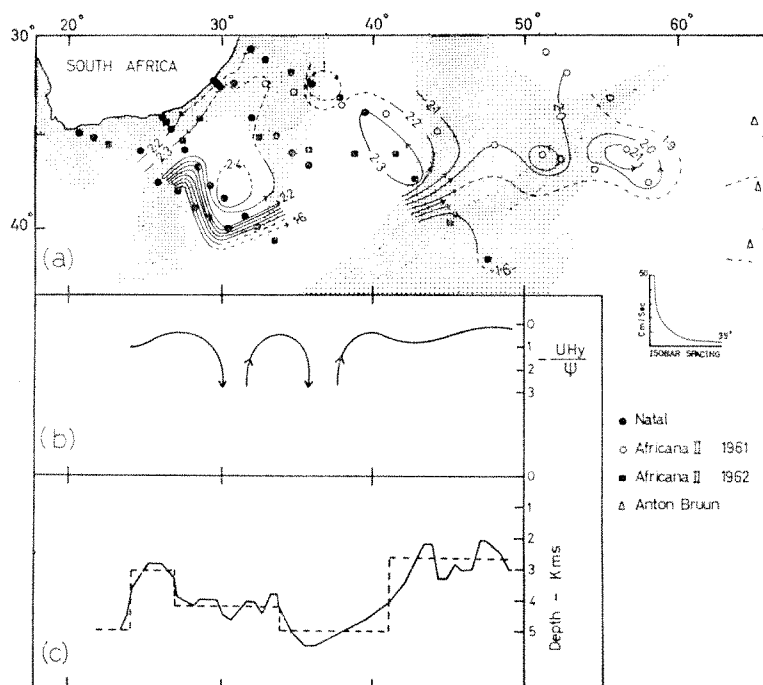


Fig. 1. a, Dynamic topography, 0/1,500 db, in dynamic metres. Bottom contours at 3,000 and 4,000 m have been shaded. b, Calculated normalized stream transport function for the barotropic mode. c, Section along 39° S showing approximate bottom topography (—) and simplified profile (---) used in the calculations. Horizontal scales for a, b and c are the same.

for the 3,000 m, water depths. Most of the essential features of the dynamic isobars are confirmed by the pattern of near surface isotherms.

Comparison of Figs. 1a and 1b which have similar horizontal scales shows that over the Agulhas Plateau both current patterns exhibit an equatorward tendency, which immediately downstream of the Plateau gives way to a sharp poleward and then equatorward deflection. The computed pattern has a phase lag which can be theoretically ascribed to the simplified form of bathymetry used. Its amplitude is, however, quite unrealistically large; although relatively large amplitudes are to be expected because the width of the Agulhas Plateau approaches a half wavelength.

Further downstream, the computed flow again deflects sharply poleward and then equatorward. The large deflection here (it is actually infinite) is caused by the increase in depth at about one wavelength distant from the Plateau. There are unfortunately no comparable hydrographic data available for this region.

Finally, over the western slope of the Madagascar Ridge both flow patterns show an equatorward deflection.

Noteworthy features indicated by the dynamic isobars are the northward branching currents, which seem to separate from the main Agulhas Return Current near the maximum equatorward deflections of the meanders. These branching currents are particularly conspicuous over the Madagascar and Indian Ridges and agree well with surface current charts based on ships' drifts¹⁰. Lacombe¹¹ and Le Pichon² have noted this tendency.

It seems that in the South West Indian Ocean there is a gyre on the west side which has an eastern limit in the region of 60°–70° E. In this vicinity, the zonal flow of the Agulhas Return Current appears from the dynamic topography to be much diminished.

Although the limitations of the dynamic topography method and the severe restrictions imposed on the mathematical model require caution in interpreting the flow patterns, it is clear from this comparative study that there are quite striking similarities between the baroclinic and barotropic modes in the Agulhas Return Flow. It is concluded that the bottom topography may well be a contributory influence on the direction of flow of this current and could account for the deflections near the Agulhas Plateau. The Agulhas Return Current provides a good opportunity for these conclusions to be tested more rigorously.

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Effect of Adhesive Tape on the Velocity Profile of Water

I REPORT here an interesting discovery, made while investigating velocity profiles close to a solid boundary. The experiments were carried out in a 5 inch channel of square section filled with water to a depth of 4.5 inches. Total head and static head tubes constructed of hypodermic tubing (0.020 inches outside diameter; 0.010 inches inside diameter) were used to measure the head by means of a 0.5 inch water gauge transducer of differential transformer type. Experiments were performed in a region of fully developed turbulent flow in order to discover the effect on the velocity profile of roughening the channel bed by sticking sand grains of various known dimensions to it by means of double-sided 'Scotch-Tape'.

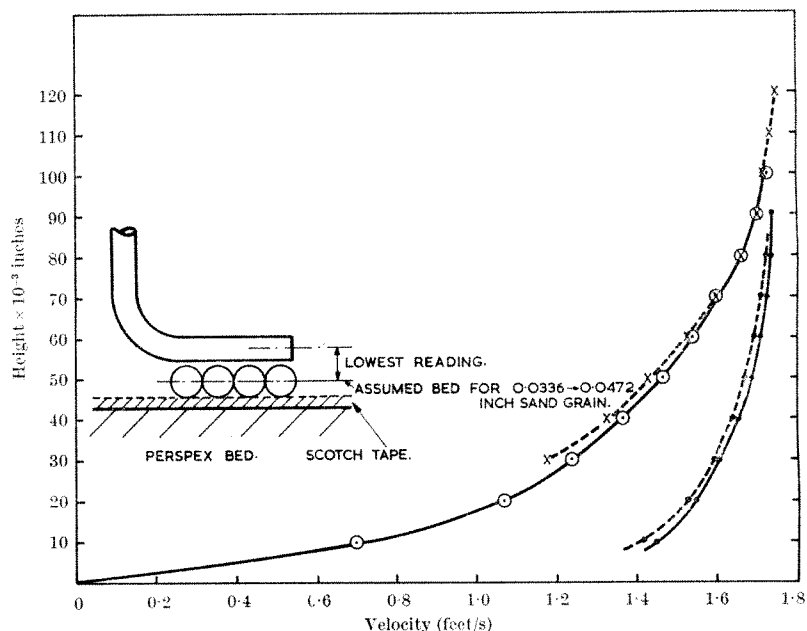


Fig. 1. Velocity profiles. ●—●, Perspex channel bed (smooth); ●---●, 'Scotch-Tape' coated with sand 0.0035–0.0059; ○—○, 'Scotch-Tape' alone; ×---×, 'Scotch-Tape' coated with sand 0.0336–0.0472.

For the larger grains of sand it was necessary to assume that the lowest point on the profile lay at half the total diameter of the head tube plus half the mean height of the roughness from the channel bed, giving the profile shown (in Fig. 1). All the velocity distributions exhibited straight lines in semi-logarithmic plots indicating typical turbulent profiles consistent with those associated with surface roughness. The fine grain sand on the 'Scotch-Tape' gave a profile which approximated to the smooth surface profile.

Out of curiosity a profile was obtained for bare 'Scotch-Tape'. When plotted, this gave the results shown in Fig. 1, which imply that the tape is physically 'sticky' towards the water; that is, the tape exhibits a 'roughness' or 'stickiness' similar to that of a roughness of mean height 0.040 inches.

I thank Professor Nonweiler and his staff in the Department of Aeronautics and Fluid Mechanics, University of Glasgow, for their help and advice.

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Microstructure of Hydrated Portland Cement Pastes

THE microstructure of calcium silicate hydrate pastes and normal portland cement, with a water to cement ratio of 0.5, has been investigated by transmission electron microscopy using Pt/C replicas. One of our chief aims was to gain a better understanding of the physical and mechanical properties of portland cement, properties thought to be affected decisively by microstructure.

It was believed that vital steps in the formation of the final microstructure of portland cement would be best revealed by studying the pure components, various combinations of these, and portland cement. For this reason the formation of the microstructure during the hydration of C_3S and C_2S paste was studied in great detail. Samples were examined following increasing times of hydration from 5 min to 6 months. One aspect of the study was to observe the effect on morphology of the admixtures, triethanolamine and calcium lignosulphonate. Another involved the etching, with glycol, of the surface of 6 month old samples of cement and C_3S and C_2S hydrated pastes to expose the underlying microstructure. Comparison was made with natural tobermorite treated similarly. Progress of the hydration reactions and the effects of etching were also followed by X-ray and electron diffraction.

Previous work has shown that the hydration products of portland cement are semi-crystalline fibres appearing as bundles, aggregates and sheets¹⁻⁷, but the relation between growth fabric and final microstructure of the hardened paste has not been clearly defined. Cleaved tabular masses observed in old cement pastes have generally been identified as calcium hydroxide. The present study indicates that the morphological evidence for this conclusion was insufficient, and that the microstructure of these platy-looking products is complicated.

In pastes of calcium silicates and portland cement, with a water to cement ratio 0.5, the initial products of hydration are amorphous-looking thin foils and fibrous particles. These products appear mainly on the surface of calcium silicate grains and grow to fill void spaces. The fibres bridge unhydrated grains and form an interlocking mesh,



Fig. 1. Electron micrograph of hydrated tricalcium silicate paste, water to cement ratio 0.5, age 1 month. Pseudomorphs surrounded by radiating fibrous hydration products intersecting at approximately 60 degree angles and merging into plates.



Fig. 2. Electron micrograph of hydrated tricalcium silicate paste, water to cement ratio 0.5, age 6 months. Platy hydration products, etched with glycol, display fibrous structure and pseudohexagonal morphology.

often meeting at an angle of about 60 degrees to form approximate isosceles triangles. Space between fibres becomes filled in with poorly crystalline hydration products composed of either a lime-rich form of calcium silicate hydrate or calcium hydroxide. Diamond⁴, using the scanning electron microscope, found that C-S-H material is encapsulated by lime or lime-rich amorphous material.

The final forms of the main hydration products are plates with marked cleavage. These frequently have a pseudohexagonal morphology, resulting from the triangular disposition of the felted fibres of calcium silicate hydrate. Surfaces of the plates often reveal the underlying fibrous structure (Fig. 1). The material that fills in space between fibres is generally more susceptible to etching by glycol than the fibres themselves (Fig. 2). It is this relation between the initial products that appear as fibres and thin sheets and the final tabular, massive structure that this paper will attempt to establish.

In the early stages of hydration, unhydrated grains of cement or calcium silicate are readily identified. When hydration is nearly complete the unhydrated grains seem to be replaced by pseudomorphs of no particular morphology (Fig. 3). These may well be of composition similar to the material that fills the space between the interlocking fibres. Cementitious material between cement pseudomorphs or grains of partly hydrated calcium silicate consists of tabular masses of platy C-S-H (Fig. 3). Because of its morphology and cleavage this has commonly been regarded as $Ca(OH)_2$.

This evidence that the C-S-H material has varying forms and morphology, and even different response to etching by glycol, leads to the conclusion that hydration products cannot be regarded as separate crystalline phases of fixed stoichiometric chemical composition but rather as solid solutions of calcium silicate hydrates and calcium hydroxide. This is in agreement with the conclusions presented by Taylor⁶ and Grutzeck and Roy⁸ showing that the Ca/Si ratio varies within the hydrated product. Morphological evidence that the hydration products are of poor crystallinity is supported by X-ray and electron diffraction data.



Fig. 3. Electron micrograph of hydrated tricalcium silicate paste, water to cement ratio 0.5, age 6 months. Main hydration product has massive tabular structure showing strong parallel cleavage. These tabular masses interconnect pseudomorphs of hydrated grains.

The morphological form of calcium silicate hydrate found in hydrated cement was the same as is present in the products of hydration of monomineral pastes. The hydrated cement is, however, much more complex because of its more variable composition.

From the standpoint of the mechanical properties of hydrated cement, the microstructure of the cementing system is really a composite material in which the fibrous network is imbedded in an amorphous material. It should provide very good mechanical properties and may well reflect the high compressive strength of the dense (low porosity) hydrated cement. It would be expected that the tensile strength of such a composite would also be high. In practice, however, it generally attains only one-tenth of the compressive strength. The work now presented shows that the masses of the cementing material appearing as tabular structures are not usually interconnected with each other but rather with areas representing original unhydrated grains (pseudomorphs, for example). Thus what might be considered interparticle connexions must be defined as material that seems to lack the reinforcement of the fibres.

This note summarizes some of the more important findings of a large-scale study and further details will be published later.

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BIOLOGICAL SCIENCES

Molecular Structure of 1-Methyl-4-thiouracil-9-methyladenine: Unusual Base Pair in tRNA

4-THIOURIDINE is a constituent of the tRNA of *Escherichia coli*¹⁻³. It is located in a position where, according to the cloverleaf model, specific base pairing by hydrogen bonding is assumed not to occur. X-ray crystallographic⁴ and spectral analyses⁵ of the monomer and polymer nucleoside have been performed and 4-thiouridine is the first pyrimidine nucleoside that has been shown to exist in a *syn* conformation in the crystalline state. We now report studies on the molecular structure of the 1-methyl-4-thiouracil-9-methyladenine complex.

We obtained yellow prismatic crystals by evaporating an aqueous solution of an equimolar mixture of the two components at room temperature. The space group, cell dimensions and density of these crystals were P1; $a = 5.777 \text{ \AA}$, $b = 12.499 \text{ \AA}$, $c = 16.481 \text{ \AA}$; $\alpha = 91.98^\circ$, $\beta = 103.98^\circ$, $\gamma = 36.94^\circ$; $\rho_{\text{obs}} = 1.442$, $\rho_{\text{calc}} = 1.457 \text{ g/cm}^3$ ($Z = 2$). We collected about 1,900 data on a Siemens four circle diffractometer (Ni-filtered Cu radiation, 2θ , ω scan mode) and corrected them for geometrical factors. The non-linearity of a Wilson plot suggested that the structure was layered. The phase problem could not be solved by application of Sayre's equation or the heavy atom method for the atoms are not evenly distributed within the unit cell and from symmetry requirements the S-S interaction is not prominent in a vector map. The structure was finally solved by superposition methods and packing considerations. After two cycles of isotropic and anisotropic and full matrix superposition methods and packing considerations. After least squares refinement, the R -factor ($R = \sum |F_o| - |F_c| / \sum |F_o|$) was found to be 8.7 per cent for the 1,344 significant reflexions ($F_o = 3.0 \sigma_{F_o}$). At this state of refinement the following features are clear.

The molecular geometry of 1-methyl-4-thiouracil and 9-methyladenine is comparable with that published for 4-thiouridine⁴, 3'-acetyl-4-thiothymidine⁶ and the 9-methyladenine-1-methylthymine complex⁷. The uracil derivative is in the keto, keto form: the C2-O2 and C4-S bond distances (1.20 Å and 1.65 Å respectively) are indicative of double bonds. The atomic arrangement of the heterocyclic rings and their substituents is nearly planar. The deviations of the substituents from the heterocycle least squares planes are less than 0.1 Å. The angle between the least squares planes of the uracil and the adenine residue in one base pair is about 5.0° .

Fig. 1 shows the crystal packing of the 1-methyl-4-thiouracil-9-methyladenine complex. The molecules are layered nearly parallel to the b , c plane at $a = 0$. The distance between adjacent layers is about 3.3 Å. The sulphur atoms of the uracil residues and the methyl groups of the adenine residues form a hydrophobic region along the a , b plane at $c = 0$. In the less hydrophobic region around $c = 1/2$, hydrogen bonds involving nitrogen and oxygen, but not sulphur, are formed. The links in a 1-methyl-4-thiouracil-9-methyladenine base pair are hydrogen bonds between N6(A) and O2(U) (2.86 Å) and between N7(A) and N3(U) (2.94 Å). The 9-methyladenine molecule is connected with its symmetry related partner by a N6(A)-N1(A) (3.00 Å) hydrogen bond.

All the crystalline adenine-uracil or adenine-thymine complexes so far investigated display the Hoogsteen type of base pairing with hydrogen bonds from adenine N6 and N7 to uracil O4 and N3. The Watson-Crick type of base pairing (adenine N6 and N1 to uracil O4 and N3) has not been observed^{7,10}. A "reversed" Hoogsteen base pairing involving uracil O2 and N3 instead of O4 and N3 has been found in this structure and in adenine-5-bromouracil complexes^{8,9}. The inductive effect of the bromine atom in the latter structures was presumed to be responsible for a less electronegative proton acceptor uracil O4 atom⁸.

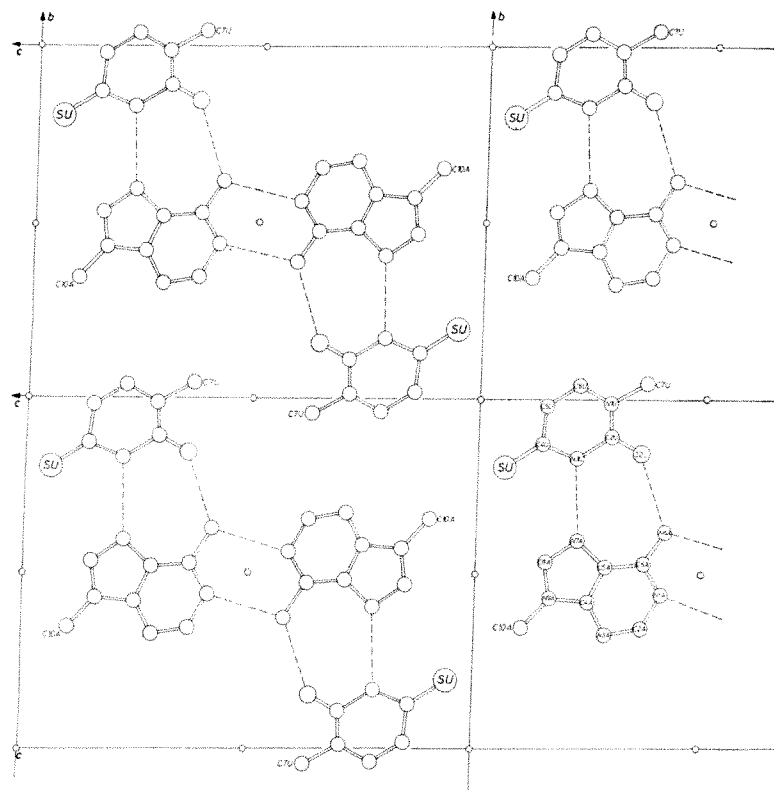


Fig. 1. Packing diagram of the 1-methyl-4-thiouracil-9-methyladenine complex projected down a^* .

This is, however, not the only effect which determines the geometry of these complexes, for in the 1-methyl-5-bromouracil-9-methyl-adenine base pair the regular Hoogsteen scheme applied¹¹. One would assume therefore that in the 1-methyl-4-thiouracil-9-methyladenine complex, the formation of a hydrogen bond involving uracil O2 instead of S4 is favoured for two reasons. The sulphur atom is less electronegative than the oxygen atom, that is, the $\text{NH} \cdots \text{S}$ hydrogen bond is weaker than an $\text{NH} \cdots \text{O}$ bond¹²; furthermore, the packing arrangement might require this type of hydrogen bonding.

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structure³ and function^{4,5} of nucleic acids. Its use is, however, complicated by the fact that it also deaminates the adenine and guanine residues in nucleic acids^{6,7}. Guanine, the reaction of which is least desirable for mutagenic purposes, is usually the base most reactive to nitrous acid⁸. In addition, nitrous acid causes the formation of nitro compounds⁹, the cross-linking of DNA (ref. 9), the destruction of guanine residues¹⁰, and other side reactions which diminish its usefulness.

We recently reported that sodium bisulphite can be used to deaminate cytosine derivatives, by the route illustrated in Fig. 1 (ref. 11). The cytosine derivative is converted quickly by sodium bisulphite, at pH 5, to an addition compound which readily deaminates, on standing, to the corresponding uracil addition compound. The latter is converted to uracil by adjustment of the pH to 9, and removal of excess bisulphite. Adenine and guanine derivatives do not react in these conditions. We now wish to report the successful application of this procedure to the specific deamination of the cytosine residues of yeast RNA.

To a solution of 5.0 mg of sodium yeast ribonucleate (Schwarz BioResearch, Orangeburg) (purified by HCl precipitation at 5° C, and washing with ethanol and ether) in 0.5 ml. of H_2O was added 118 mg of NaHSO_3 . The pH was adjusted to 5 by addition of concentrated HCl, and the reaction was allowed to proceed as indicated in Table 1.

The pH was then adjusted to 9 with 1 M NaOH. The RNA was separated from the bisulphite by chromatography on 'Sephadex G-25M' (Pharmacia, Piscataway). For analysis of composition, the RNA was hydrolysed at 37° C with 10 ml. of 1 M KOH for 24 h. The solution was neutralized to pH 6.5 with HClO_4 , cooled at 4° C for 16 h, and the precipitated KClO_4 was removed by filtration. The nucleotide mixture was then fractionated on a column of Amberlite 'CG-400' resin (formate), using an exponential gradient in formate (for details see ref. 12). The column is capable of resolving the four major natural ribonucleoside 2'-(3')-phosphates, as well as inosine 2'-(3')-phosphate and xanthosine 2'-(3')-phosphate. The peaks eluted were identified by their position and by their R_f (thin-layer chromatography) in a solvent made of 13.8 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ dissolved in 900 ml. of H_2O . The pH of this solvent was adjusted to 6.8 with H_3PO_4 , the volume was diluted to 1,000 ml. with H_2O , and 200 g of $(\text{NH}_4)_2\text{SO}_4$ and 20 ml. of 1-propanol were added. The R_f values observed for the 2'-(3')-phosphates of adenosine, guanosine, uridine and cytidine, respectively, were 0.31, 0.52, 0.76 and 0.80. The quantitative amounts of each

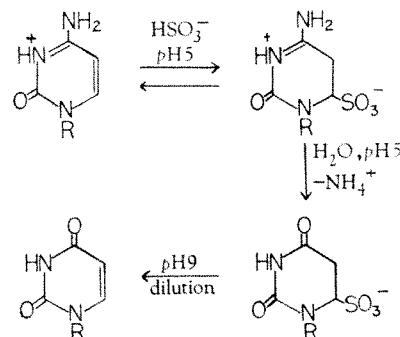


Fig. 1. Deamination of cytosine derivatives by bisulphite

Specific Deamination of RNA by Sodium Bisulphite

THE ability of nitrous acid to deaminate the cytosine residues of nucleic acids to uracil has made it valuable both as a mutagen^{1,2} and as an aid to studies of the

peak eluted from the column were determined by a spectrophotometric method¹².

The results of four deamination runs, in various conditions, are summarized in Table 1. No new reaction products were observed, and all of the adenine and guanine nucleotides were recovered (within experimental error). A varying amount of deamination of the cytosine residues of the RNA to uracil was observed. An initial portion of the cytosines reacted rapidly. The rate of reaction then slowed down, and finally levelled off when 90 per cent of the cytosines was deaminated. This is not surprising, considering the heterogeneous nature of yeast RNA. It suggests that the reactivity of cytosine residue to bisulphite is strongly influenced by the secondary structure of the RNA. The altered composition of the RNA in run 4 was reflected in its ultraviolet spectrum. The ratio A_{280}/A_{260} for the deaminated RNA (pH 2) was 0.44 ($\lambda_{\max}=258$) whereas the corresponding ratio for the starting RNA was 0.62 ($\lambda_{\max}=260$).

Table 1. ANALYSIS OF BISULPHITE-MODIFIED RNA

Run	Conditions	Nucleotides isolated (mmoles $\times 10^3$)				Deamination of Cp (per cent)
		Cp	Up	Ap	Gp	
1	Unmodified RNA	2.3	3.1	2.8	3.2	
2	15 h, 25° C	1.3	4.3	2.5	3.1	45
3	24 h, 37° C	0.75	4.8	2.5	3.2	68
4	84 h, 37° C	0.20	5.2	2.7	3.3	91
5	168 h, 37° C	0.21	5.7	2.9	3.4	92

The abbreviations Cp, Up, Ap, and Gp refer to the 2'-(3')-phosphates of cytidine, uridine, adenosine, and guanosine, respectively.

Preliminary experiments with DNA confirm the dependence of cytosine reactivity on nucleic acid secondary structure. In the conditions of run 5, the cytosines of single stranded phage F₁ DNA were completely converted to uracils, while those of double stranded calf thymus DNA were essentially unreacted. Thus sodium bisulphite should be of value for the chemical modification of nucleic acids, and may replace nitrous acid in many of its uses.

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Inhibition of Vaccinia Virus Growth by 1-Methyl-4-aminopiperazine

RIFAMPICIN inhibits the replication of poxvirus^{1,2}; experiments with other rifamycin derivatives suggested that the hydrazone side-chain of rifampicin was essential for its antiviral activity³. We describe here the action of the characteristic component of this side-chain, 1-methyl-

4-aminopiperazine, and compare it with that of the other analogue of rifampicin, 3-formylrifamycin SV (FSV), and another compound, 1-methyl-4-benzylidenaminopiperazine, which contains the hydrazone side-chain but not the rifamycin moiety.

The activities of these drugs, together with twenty-seven other rifamycin derivatives, were screened by plating eighty plaque forming units (p.f.u.) of various viruses on primary cultures of chick embryo fibroblasts. After the adsorption period, the cell sheet was overlaid with nutrient medium in agar containing 800–100 μ g/ml. of each drug. With vaccinia virus, methylaminopiperazine and methylbenzylidenaminopiperazine completely inhibited plaque formation at 200–300 μ g/ml. The second drug was slightly toxic to the cells at 800 μ g/ml. At twice the limiting dose of toxicity, none of the drugs inhibited plaque formation by fowl plague, Newcastle disease, pseudorabies and herpes type 2 viruses. Some of the drugs were highly toxic: the cells did not stain with neutral red after 4 days' treatment with 50–100 μ g of 4-benzylidendi-azo-4-deoxyrifamycin S (BDS), the O-benzoyloxime of 3-formylrifamycin SV (BOF), 4-(1-methyl-2-imidazolin-2-ylidiao)-4-deoxyrifamycin SV (MID) or 1'-benzyl-2'-methyl-3'-acetylpyrrolo-(3,2-C)-4-deoxyrifamycin SV (BAP).

In cells treated for 1 h with 200–400 μ g/ml. of these compounds ¹⁴C-uridine incorporation was inhibited by 90 per cent, and the incorporation of a hydrolysate of ¹⁴C-protein was only reduced by 40–50 per cent. Methylbenzylidenaminopiperazine also inhibited uridine incorporation but its action was more easily reversed by washing the cells; methylaminopiperazine had no effect. Similar results were obtained when *in vitro* transcription with sonicated nuclei was investigated (Table 1).

Although the activity of polymerase aggregate was not influenced by methylaminopiperazine in the assay mixture *in vitro*, treatment of whole cells with methylamino-

Table 1. ACTION OF VARIOUS INHIBITORS (400 μ g/ml.) ON INCORPORATION OF ³H-UTP INTO ACID PRECIPITABLE MATERIAL OF SONICATED NUCLEI FROM CHICK EMBRYO FIBROBLASTS

	Incorporation (d.p.m.)	
	Number	Per cent of control
No drug	1,424	
BDS	348	24
MID	191	13
FSV	1,508	106
Rifampicin	1,291	90
Methylaminopiperazine	1,928	135
Methylbenzylidenaminopiperazine	178	12
BAP	522	36
BOF	624	44

The components of the assay mixture were added in the following order: (a) 0.7 ml. of a solution containing 80 μ moles of Tris-HCl (pH 8.6), 3.5 μ moles of MgCl₂, 1 μ mole each of CTP and GTP, 2 μ moles of ATP, 0.1 μ mole of UTP, 5 μ moles of phosphoenolpyruvate and 0.01 M mercaptoethanol; (b) 400 μ g of one inhibitor; (c) 2 μ Cl (corresponding to 0.0013 μ mole) of ³H-UTP and 20 μ g of pyruvate kinase; (d) 0.3 ml. of sonicated nuclei obtained from 8×10^7 cells, according to the method of Bach and Johnson⁴. After incubation for 30 min at 37° C, trichloroacetic insoluble radioactivity was determined by standard procedures.

Table 2. ACTION OF METHYLAMINOPIPERAZINE ON WHOLE CELLS AND ON BROKEN CELLS

	Incorporation of ³ H-UTP by sonicated nuclei (d.p.m.)	
	Number	Per cent of control
No inhibitor	2,898	
Methylaminopiperazine (800 μ g/ml.)		
(a) On suspended whole cells, for 2 h at 37° C	928	32
(b) On suspended whole cells, for 30 min at 37° C	522	18
(c) On suspended whole cells, for 30 min at 0° C in distilled water	456	16
(d) On broken cells, immediately after treatment with the Dounce homogenizer	2,745	95
(e) On the enzyme aggregate, in the assay mixture before addition of ³ H-UTP	2,650	95

5×10^6 Embryo fibroblasts were suspended in Eagle's medium without calcium and with 0.5 per cent glutamine plus 2.5 per cent calf serum. The suspension was divided into six aliquots which were agitated for 2 h at 37° C; samples (a) and (b) were treated with the drug for 2 h and the last 30 min, respectively. All the samples were then centrifuged and the sediments collected in ice-cold distilled water. The drug was then added to sample (c); after 30 min at 0° C and treatment with 0.25 per cent Triton X-100, the cells were disrupted with six strokes of a Dounce homogenizer and the drug was added to sample (d). The nuclei were isolated, sonicated, and assayed for polymerase activity as in Table 1. In one mixture (e), the drug was added just before ³H-UTP.

piperazine before preparation of the nuclei extract resulted in the suppression of transcription activity of this extract; the effect was lower after 2 h than after 30 min of treatment at 37° C, an indication of the transient presence of the drug in its active form (Table 2). The drug seems not to prevent the synthesis of polymerase but, rather, to bind to some component (polymerase?) during the preparation of the nuclear extract. A similar inhibition of transcription was obtained, whether the cells were pre-incubated for 30 min at 37° C with the drug, or whether they were only in contact with the drug when they were allowed to swell in distilled water for 30 min at 0° C. By contrast, the "site" susceptible to methylaminopiperazine was apparently no longer available when the structure of the cell was destroyed, for addition of the drug after homogenization with the Dounce apparatus did not cause any inhibition of transcription.

Methylaminopiperazine did not inhibit *in vitro* RNA synthesis by 2 units of *E. coli* RNA polymerase in the presence of 100 µg/ml. thymus DNA. By contrast, BDS (4 µg/ml.) inhibited this reaction by 73 per cent; BOF and MID also had some activity. Methylbenzylidenaminopiperazine was not active on the bacterial polymerase, although it inhibits transcription by the enzyme aggregate from chick embryo fibroblasts.

We now return to the action of methylaminopiperazine on vaccinia virus replication. Its effect in liquid medium, on several cycles of growth, is smaller than that described for solid medium. After treatment for 21 h with 200, 50 and 20 µg/ml. of methylaminopiperazine, the virus titres were 24, 40 and 100 per cent of the controls, respectively. Inhibition of virus growth, however, was 86 per cent if three doses of 20 µg/ml. each were given to the cells at 6 h intervals. The action of the drug thus seems transient.

Because of the reversibility of the action of methylaminopiperazine, the time of its action was shown in the

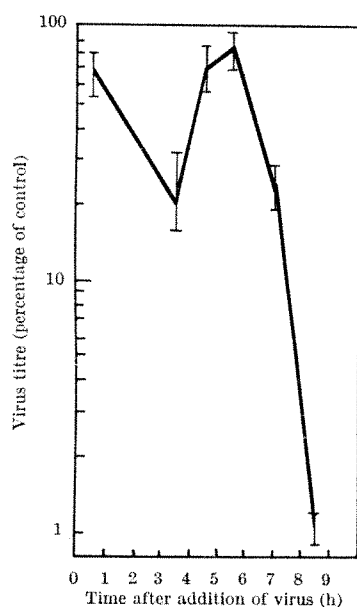


Fig. 1. Single-step growth cycle of vaccinia virus in monolayer of chick embryo fibroblasts, with 1 h pulse treatments by 500 µg/ml. methylaminopiperazine. Unadsorbed virus was washed from all the cultures 3 h after the addition of virus; each 1 h treatment with drug was also followed by washing and addition of fresh nutrient medium. A control series of cultures received no drug but received fresh medium at the same times. For all cultures, virus was titrated in the disrupted cells 12 h after addition of the virus. The points in the graph correspond to the 1 h drug treatment and are the means of three experiments, expressed in percentage of the virus titre for control cultures.

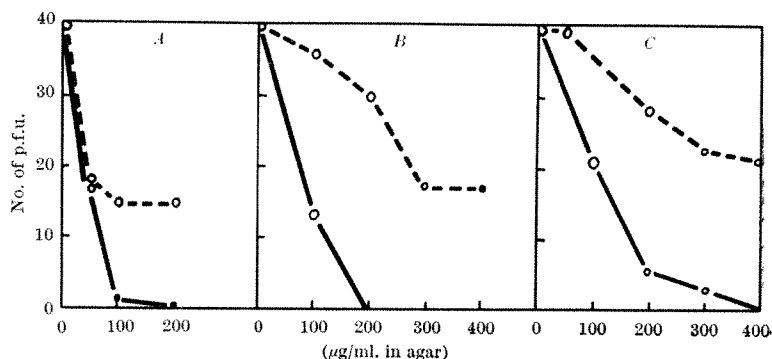


Fig. 2. Number and size of vaccinia virus plaques, with various concentrations of rifampicin (A), methylaminopiperazine (B) or methylbenzylidenaminopiperazine (C) in agar (—), or with these drugs plus 10 µg/ml. FSV (---). ○, Plaques of normal size; ○, small plaques; ●, minute plaques.

conditions of a single-step growth cycle (Fig. 1). The early and the late period of the cycle of growth are susceptible to a 1 h treatment with 500 µg/ml. of the drug, but the processes which take place 4 to 6 h after addition of the virus are completely resistant.

It was noted that FSV at low doses (10 µg/ml.) decreases the inhibitory effect of methylaminopiperazine, methylbenzylidenaminopiperazine and rifampicin on vaccinia virus plaques (Fig. 2).

HeLa cells were grown in suspension and infected with 10–20 p.f.u. of vaccinia virus per cell; cytoplasmic extracts were prepared 6 h later and assayed for polymerase activity with no DNA added; there was no ³H-UTP incorporation with cytoplasmic extracts from uninfected cells, and the activity of the viral enzyme, in the cytoplasm, was not inhibited by methylaminopiperazine; it was, however, completely blocked by BDS (Table 3, experiment 1). Thus the RNA polymerase bound to viral DNA is not completely masked, for it is susceptible to BDS, a drug which therefore seems to have a broad range of action: it has also been shown to inhibit both bacterial and animal cell polymerases.

Table 3. EFFECT OF INHIBITORS ON VACCINIA VIRUS RNA POLYMERASE PREPARED FROM CYTOPLASMIC FRACTION OF INFECTED CELLS

Expt.	Inhibitors added	Incorporation of ³ H-UTP (d.p.m.)
1	<i>In vitro</i> to the assay mixture	
	No drug	800
	BDS	0
2	To whole cells, 30 min before preparation of cytoplasmic extract	
	No drug	1,046
	Methylaminopiperazine	500

Cytoplasmic fractions were prepared according to Kates *et al.*¹ from HeLa cells infected for 6 h with vaccinia virus; the fractions were assayed for RNA polymerase activity as described in the legend to Table 1. In experiment 1, 400 µg/ml. of BDS or 800 µg/ml. of methylaminopiperazine were added to the assay mixture immediately before addition of ³H-UTP. In experiment 2, 800 µg/ml. of methylaminopiperazine were added to the cell suspension 5–5 h after infection with the virus; the drug was left in contact with whole cells for 30 min at 37° C before preparation of the cytoplasmic extract.

In a second experiment, HeLa cells infected with vaccinia virus for 5.5 h were treated with 800 µg/ml. methylaminopiperazine for 30 min at 37° C and the cytoplasmic fraction was then immediately prepared. In these conditions, the drug inhibited RNA polymerase activity of the fraction by 50 per cent (Table 3, experiment 2). Thus, like rifampicin⁶, methylaminopiperazine has no action on the *in vitro* activity of vaccinia virus RNA polymerase, but prevents at least partly its action when added to whole cells.

We then attempted to obtain mutants resistant to some of the drugs. Vaccinia virus was grown in liquid medium in chick embryo fibroblasts treated with 200 µg/ml. of methylaminopiperazine or methylbenzylidenaminopiperazine, 100 µg/ml. of rifampicin, or 20 µg/ml. of BDS or FSV. After three passages in the same concentration of drugs, the populations of viruses were titrated in the presence or absence of the corresponding drug in agar

Table 4. ASSAY FOR SUSCEPTIBILITY TO DRUGS OF VARIOUS VACCINIA VIRUS POPULATIONS GROWN FOR THREE PASSAGES IN THE PRESENCE OF DRUGS

Virus grown with	Titrated with the following drugs in agar (doses in $\mu\text{g}/\text{ml}$)					
	Methylamino- piperazine		Methylbenzylidenamino- piperazine		Rifampicin	
BDS	300	200	300	200	200	150
Rifampicin	0	25 s	0	20 s	0	25 s
Methylaminopiperazine	30 s	60 n	66 s	80 s	66 n	60 n
Methylbenzylidenaminopiperazine	40 s	70 s	40 s	100 n	66 s	50 s
No drug	0	28 s	0	18 s	0	20 s

Values represent percentage plaques calculated from the number of plaques obtained with no drug in the agar. s and n: plaques of small and normal size. Viruses grown with FSV or methylbenzylidenaminopiperazine showed similar susceptibilities as viruses grown with BDS or rifampicin, respectively.

(Table 4). The inhibition of plaque formation by methylaminopiperazine, methylbenzylidenaminopiperazine or rifampicin for viruses previously cultivated in the presence of BDS or FSV, was similar to that for the control viruses grown without drugs. By contrast, all three drugs in agar were less active on viruses cultivated with rifampicin, methylaminopiperazine, or methylbenzylidenaminopiperazine. Cloned stocks grown from thirty plaques did not prove to be clearly resistant to the drugs, however, by contrast with previous reports of rifampicin-resistant mutants². The results in Table 4 indicate, however, that virus populations with cross resistance to rifampicin, methylaminopiperazine and methylbenzylidenaminopiperazine can be obtained. Our other observations also support the hypothesis that methylaminopiperazine and rifampicin inhibit the replication of vaccinia virus by the same mechanism.

Compounds MID and BDS were synthesized by Dr N. Maggi (methods to be reported elsewhere). We thank Mr Léopold Tack and Jacques Stiénon for technical assistance.

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Reversal of Anti-viral Effects of Rifampicin

RIFAMPICIN prevents vaccinia virus growth^{1,2} by a still undefined mechanism. Viral DNA and early and late species of messenger RNA are synthesized in the presence of rifampicin³⁻⁶; early and late viral proteins are also made, although synthesis of the latter declines sooner than in the absence of the drug^{3,4,6,7}. Rifampicin does not inhibit the RNA polymerase activity contained within purified vaccinia virus particles^{3,5,7-9}, but the drug prevents the formation of particulate RNA polymerase activity⁵. This enzyme can ordinarily be demonstrated, at late times in vaccinia-infected cells, after treatment of cytoplasmic fractions with mercaptoethanol and detergents¹⁰⁻¹³. Rifampicin has also been found to block vaccinia virus assembly during the formation of the viral envelope and preceding the appearance of virus particles^{6,14}. The sequence of events which follows the removal of rifampicin has now been examined in order to correlate

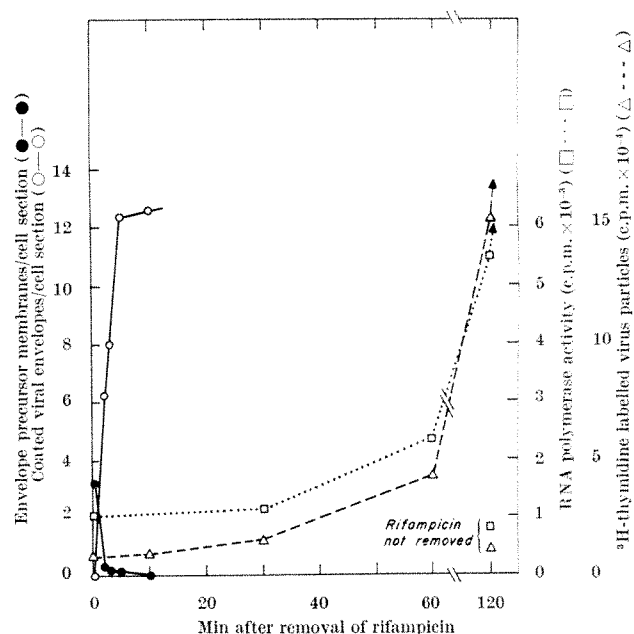


Fig. 1. The sequence of events following the removal of rifampicin. The number of membrane limited regions of viroplasm (envelope precursor membranes) were counted in 100 HeLa cell thin sections and compared with the total of coated envelope profiles formed after the removal of rifampicin. Cytoplasmic extracts were prepared by disrupting cells in 0.01 M Tris-HCl buffer, pH 8.6. The nuclei were removed by centrifugation at 500g for 2.5 min and the supernatant was treated with 0.05 per cent Nonidet P-40 detergent (Shell Chemical Co., New York) and 0.02 M dithiothreitol at 0°C for 30 min. RNA polymerase activity was measured by the incorporation of ³H-UTP into acid precipitable material⁵. The formation of ³H-thymidine labelled viral particles was determined as previously described⁶ in a separate experiment under similar conditions. The two points in the lower right are control values obtained when rifampicin was not removed.

the effects observed on the formation of the late particulate RNA polymerase activity and on the assembly of viral envelopes.

HeLa cells were treated with rifampicin (100 $\mu\text{g}/\text{ml}$) 10 min before infection with vaccinia virus (thirty plaque forming units/cells)³. After 8 h, the drug was removed by washing the cells three times with ice-chilled medium. The cells were then resuspended in warm medium (0 time) and the incubation was continued at 37°C. At intervals, samples were removed and rapidly chilled by pouring over crushed frozen medium. The cell pellet was fixed with cold glutaraldehyde and prepared for electron microscopy⁶. Total cytoplasmic RNA polymerase activity in another portion was measured by a procedure similar to that of McAuslan⁵.

Except for rare immature vaccinia particles, the only viral structures which could be defined by electron microscopy in the rifampicin treated cells consisted of numerous irregular membranes which surrounded portions of electron dense viroplasm^{6,14}. These irregular membranes were trilaminar, but lacked the outer spicule coat which is characteristic of poxvirus envelopes. The irregular membranes were direct precursors of virus envelopes as shown by the rapid appearance of transitional coated forms after removal of rifampicin¹⁴. Each precursor envelope structure seems to give rise to several coated envelopes⁶. Conversion of envelope precursor membranes to coated envelopes was almost complete within 10 min after rifampicin was removed, and the number of the latter rose dramatically during this time (Fig. 1). An appreciable increase in the late particulate RNA polymerase activity could not be demonstrated until after the rifampicin effect on the formation of the viral envelope was completely reversed (Fig. 1). The rise in polymerase activity was nearly coincident with the formation of DNA-containing virus particles isolated by sucrose density gradient centrifugation and may signify that integration into

particulate form is required before this enzyme activity can be detected.

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Immunoglobulin Allotypic Determinants on Rabbit Lymphocytes

THE initiation of the immune response is believed to involve the recognition of antigen by specific receptors on the surface of lymphocytes. The transformation of rabbit and human lymphocytes by antisera to immunoglobulins¹⁻³, the blocking of antigen binding by lymphocytes using anti-immunoglobulins⁴ and the inhibition of antigen induced lymphocyte transformation by anti-light chain serum⁵ suggest that such receptors are immunoglobulin in nature or at least possess immunoglobulin determinants. We have now obtained direct evidence for the presence of immunoglobulin determinants on the surface of rabbit lymphocytes by autoradiography using radio-labelled anti-allotype sera.

Anti-allotype sera were raised against allotypes Ab4 and Ab5 by immunizing rabbits of one allotype with antibody of another allotype coated on to killed *Proteus vulgaris*⁶. The sera were monospecific with respect to the allotypes Aa1, 2 and 3 and Ab4, 5 and 6 as judged by gel precipitation. The IgG fractions of these antisera and of the sera of normal rabbits of allotypes Ab4,4 and Ab5,5 were obtained by chromatography on DEAE-cellulose in 0.03 M phosphate buffer (pH 6.5). Labelling of the IgG with ¹²⁵I was performed by the chloramine T method^{7,8}: 1 mCi of ¹²⁵I (specific activity 8-12 Ci/mg; Radiochemical Centre, Amersham: 'IMS4') was mixed with 500 µg of protein in phosphate-buffered saline (PBS) at room temperature and chloramine T added to a final concentration of 450 µg/ml. After 1 min the reaction was terminated by the addition of sodium metabisulphite at a final concentration of 1.2 mg/ml. Inorganic iodide and other low molecular weight products were removed from the iodination mixture by passage through 'Sephadex G25' previously equilibrated in PBS.

Peripheral blood was obtained from three rabbits of genotypes Ab4,4, Ab5,5 and Ab4,5. Red blood cells were sedimented in 3.5 per cent dextran 'T250' (Pharmacia) in 0.9 per cent saline at 37° C for 45 min. The leucocyte-rich supernatant was spun down at 150g and the cell

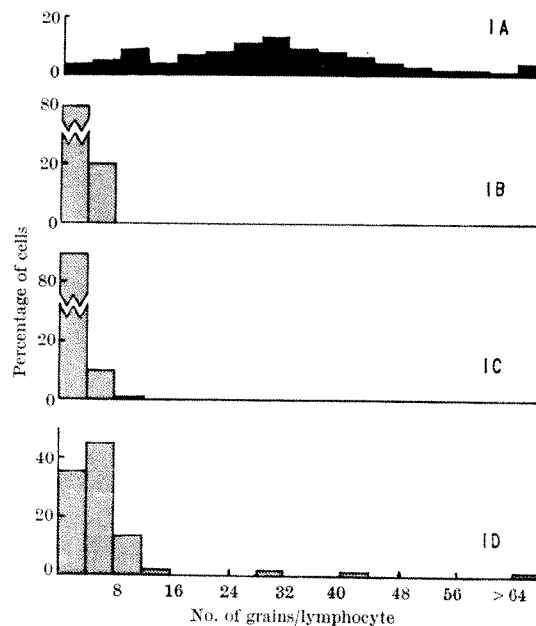


Fig. 1. Frequency distributions of grain density (grains/cell) on the lymphocytes from the homozygous Ab 4.4 rabbit. 1A, Treated with labelled anti-4; 1B, 1C and 1D, controls, see Table 1. Cells with a grain density greater than eleven were considered to be specifically labelled.

pellet washed three times in Eagle's medium at 4° C. Cell counts were performed conventionally in 2 per cent acetic acid and aliquots containing 2×10^6 lymphocytes obtained. Each aliquot was spun down and re-suspended in 0.25 ml. of iodinated antibody at a concentration of 600 µg/ml. in PBS for 30 min at 4° C. The details of specific and control incubations are shown in Table 1. After incubation with antibody the cells were spun down and washed four times in Eagle's medium at 4° C, and rich supernatant was spun down at 150g and the cell (40g, 15 min). The smears were fixed in methanol and dipped in a 1:4 dilution of Ilford 'K5' nuclear emulsion to give an approximate emulsion thickness of 2-3 µm. Slides were exposed for 20 days, developed in Kodak 'D19b' and stained with May-Grünwald-Giemsa. After coding the slides, grain counts on approximately 200 lymphocytes (100 for each of two slides) were performed for each of the numbered experiments.

Table 1. SPECIFIC AND CONTROL INCUBATIONS OF LYMPHOCYTES WITH LABELLED ANTIBODY

Cell genotype	Experiment	Reagents added
Ab 4,4	1 A	¹²⁵ I-anti-Ab 4*
	1 B	¹²⁵ I-anti-Ab 5
	1 C	Excess anti-Ab 4, then ¹²⁵ I-anti-Ab 4
	1 D	Excess Ab 4 + ¹²⁵ I-anti-Ab 4
Ab 4,5	2 A	¹²⁵ I-anti-Ab 4
	2 B	¹²⁵ I-anti-Ab 5
	2 C	Excess anti-Ab 4, then ¹²⁵ I-anti-Ab 4
	2 D	Excess anti-Ab 5, then ¹²⁵ I-anti-Ab 5
	2 E	Excess Ab 4 + ¹²⁵ I-anti-Ab 4
	2 F	Excess Ab 5 + ¹²⁵ I-anti-Ab 5
Ab 5,5	3 A	¹²⁵ I-anti-Ab 5
	3 B	¹²⁵ I-anti-Ab 4
	3 C	Excess anti-Ab 5, then ¹²⁵ I-anti-Ab 5
	3 D	Excess Ab 5 + ¹²⁵ I-anti-Ab 5

* Ab 4, anti-Ab 4 and so on refer to IgG fractions of specific allotype or antibody directed against allotypic determinants.

† Excess of Ab 4 and anti-Ab 4 and so on was ten-fold relative to labelled anti-Ab 4. Treatment of cells with unlabelled antibody preceded the addition of the labelled antibody by 30 min.

Lymphocytes showed specific labelling with the appropriate anti-allotype (experiments 1A, 2A, 2B and 3A), a wide range of grain densities being observed (Figs.1-4). There was some overlap with the results obtained in the controls and lymphocytes were only regarded as specifically labelled if the grain density exceeded that in the controls (see legends). In the homozygous rabbits the number of positively labelled lymphocytes was 83 per cent in the

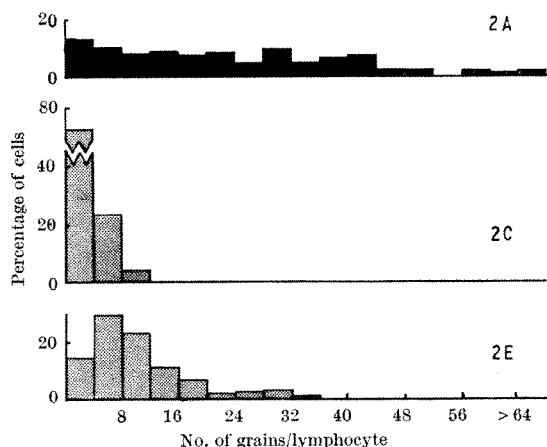


Fig. 2. Frequency distributions of grain density on the lymphocytes from the heterozygous Ab 4,5 rabbit. 2A, Treated with labelled anti-4; 2C and 2E, controls, see Table 1 and text.

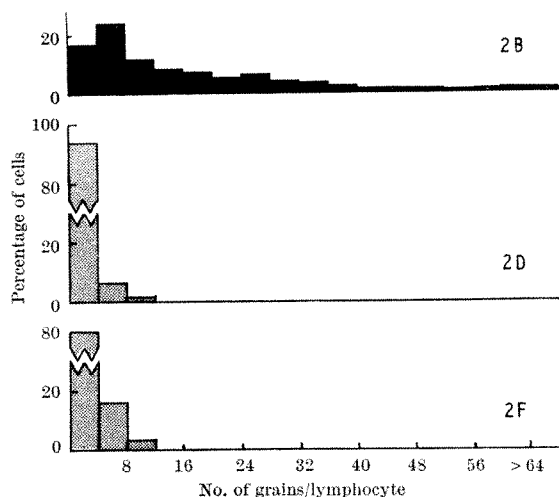


Fig. 3. Frequency distributions of grain density on the lymphocytes from the heterozygous Ab 4,5 rabbit. 2B, Treated with labelled anti-5; 2D and 2F, controls, see Table 1. Cells with a grain density greater than 11 were considered to be specifically labelled.

Ab4,4 animal (experiment 1A) and 72 per cent in the Ab5,5 animal (experiment 3A). In the heterozygous Ab4,5 animal 30–50 per cent of the lymphocytes labelled with anti-allotype 4 (experiment 2A), depending on the control baseline accepted, and 47 per cent with anti-allotype 5 (experiment 2B). The appearance in the light microscope suggested surface labelling of the lymphocytes and this was confirmed in a separate experiment by electron-microscopic autoradiography (Fig. 5a and b). Red cells, monocytes and the majority of polymorphs had grain densities within the range of control preparations. A few blast-like cells (less than 1 per cent of the total white cells) were heavily labelled. Comparable results were obtained with three further Ab4,4 rabbits where 91, 85 and 80 per cent respectively of the blood lymphocytes were specifically labelled with anti-Ab4.

The results are consistent with the existence on rabbit lymphocytes of membrane components having specific allotypic determinants. It is unlikely that the labelling observed was the result of phagocytosis or antibody secretion because experiments were carried out at 4° C. The fact that only 40–50 per cent of the cells were labelled in the heterozygote rules out the possibility of non-specific adsorption of IgG from the donor serum; if cytophilic antibody were present one would expect 100 per cent of the lymphocytes in the heterozygote to be labelled with each anti-allotype. Because approximately 85–90 per cent of

the serum immunoglobulins in the homozygote carry the *b* locus allotype⁸, our finding of these allotypic markers on 70–85 per cent of the lymphocytes makes it likely that nearly all peripheral blood lymphocytes in the rabbit will be shown to possess surface immunoglobulins. Thus the labelling of only 40–50 per cent of the lymphocytes in the heterozygote by each anti-allotype would suggest that individual cells carry only one allotypic marker. Direct evidence for allelic exclusion has been obtained in the case of plasma cells and splenic small lymphocytes using double-labelling immunofluorescent techniques (ref. 10 and unpublished results of B. Pernis).

Compared with other direct methods for demonstrating allotypic markers on rabbit peripheral blood lymphocytes, autoradiography seems to be the most sensitive. In our experiments up to 83 per cent of the lymphocytes were labelled, but not more than 50 per cent stain with fluorescent labelled anti-allotype (unpublished results of B. Pernis), and only 60 per cent form rosettes with anti-allotype-coated erythrocytes¹¹.

The percentage of mouse lymphocytes bearing surface markers is not as high as in rabbits. Raff *et al.*¹², using autoradiographic and fluorescent techniques, found that only 40 per cent of mouse splenic lymphocytes labelled with anti-IgG, although they noted a difference in the sensitivity of the two techniques; our own preliminary experiments using autoradiography are consistent with these findings in that only 55 per cent of mouse peripheral blood lymphocytes labelled with anti-light chain.

In these studies with rabbit lymphocytes, each grain corresponds approximately to 700 bound anti-allotype molecules assuming an efficiency of 50 per cent for iodination and 10 per cent for autoradiography. On this basis the number of anti-allotype molecules attached to each cell varied from 7,000 to more than 40,000. This implied variation in number of surface receptors per cell may depend on the immunoglobulin class, the age of the lymphocytes and perhaps their immunological function.

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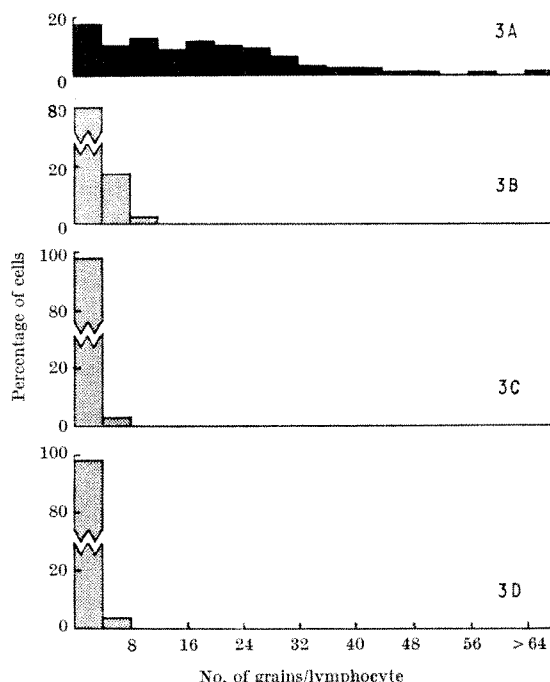


Fig. 4. Frequency distributions of grain density on the lymphocytes from the homozygous Ab 5,5 rabbit. 3A, Treated with labelled anti-5; 3B, 3C and 3D, controls, see Table 1. Cells with a grain density greater than 7 were considered to be specifically labelled.

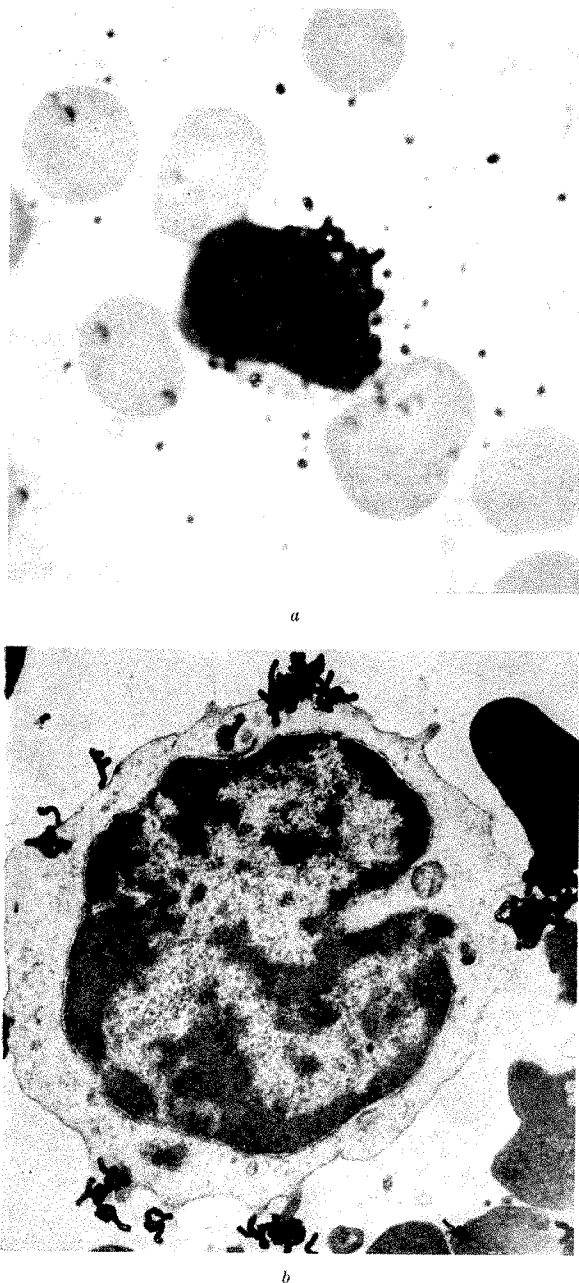


Fig. 5. Specifically labelled lymphocytes (a) under light microscopy, (b) electron microscopy.

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Effect of Diameter on the Electrical Constants of Frog Skeletal Muscle Fibres

THE relatively large electrical capacity of skeletal muscle fibres^{1,2} is generally attributed to the transverse tubular system which ramifies over the cross-section of the fibre and enlarges the effective area of the surface. From an analysis of the frequency dependence of the impedance, Falk and Fatt³ assigned a capacity of $2.6 \mu\text{F}/\text{cm}^2$ to the surface membrane and $4.1 \mu\text{F}/\text{cm}^2$ to the contribution of the tubules. Gage and Eisenberg⁴ showed that fibres "detubulated" by the glycerol method had a low frequency capacity of $2.1 \mu\text{F}/\text{cm}^2$ as compared with $5\text{--}12 \mu\text{F}/\text{cm}^2$ in normal fibres.

If the capacity resided wholly in the surface the quantity C_M (the apparent capacity per unit area of surface), would be expected to be independent of diameter. On the other hand, if the capacity were chiefly tubular in origin, C_M should increase linearly with fibre diameter provided current spreads to the middle of the fibre. The experiments described here were designed to test this prediction and to obtain more reliable estimates of the internal conductivity.

Single fibres were isolated from the sartorius or semitendinosus muscles of *Rana temporaria*. The diameter was determined by measuring the thickness in two directions at right angles at about seven points along the length used in the electrical measurements. Two microelectrodes were inserted; one applied rectangular current pulses lasting about 300 ms in inward and outward directions; the other recorded the electrotonic potential at varying distances from the current electrode. The method of analysis was similar to that used previously^{1,2,5}, except that allowance was made for the finite length of the fibres. The Q_{10} of the sarcoplasmic conductivity was 1.37 and of the membrane conductance 1.49, and these values have been used to correct for small variations in temperature between experiments. It was unnecessary to correct C_M since the Q_{10} of the capacity was close to unity.

Twenty-one isolated twitch fibres with diameters varying between 38 and 165 μm were measured at room temperature. The results (Fig. 1) show that the sarcoplasmic conductivity G_i did not vary with fibre diameter and at 20°C was $5.91 \pm 0.13 \text{ mmho}/\text{cm}$ (mean and s.e., $n=21$). By contrast, the low-frequency membrane capacity per unit area of surface increased from about $3 \mu\text{F}/\text{cm}^2$ at 40 μm diameter to about $10 \mu\text{F}/\text{cm}^2$ at 160 μm .

Optical measurements of fibre diameter are not reliable in whole muscle or in bundles of fibres. But because the experiments with isolated fibres showed that sarcoplasmic conductivity is independent of fibre diameter, a diameter can be obtained from the internal resistance per unit length and the mean value of the sarcoplasmic conductivity at the temperature of the experiment.

The upper set of points in Fig. 2 gives the low frequency capacity (C_M), measured with rectangular current pulses, as a function of diameter in fibres in the sartorius muscle or in bundles from the semitendinosus. The results, which are essentially the same as in Fig. 1, again show that C_M increases with diameter in an approximately linear manner. The average C_M of the fibre group with diameters between 120 and 139 μm was $8.5 \pm 0.19 \mu\text{F}/\text{cm}^2$ (mean and s.e., $n=19$) whereas that in the range 40 to 59 μm was $4.6 \pm 0.17 \mu\text{F}/\text{cm}^2$ ($n=9$).

The values of the membrane conductance G_M also in-

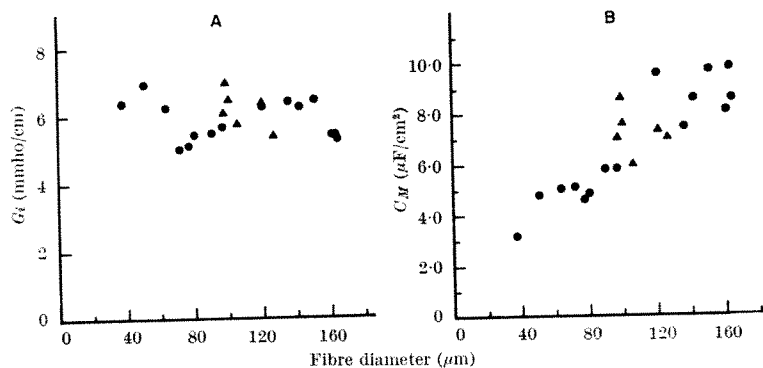


Fig. 1. Internal conductivity, G_i , versus fibre diameter (A), and low frequency membrane capacity, C_M , versus fibre diameter (B) in isolated twitch fibres. ●, Semitendinosus. ▲, sartorius. Experiments were carried out at 20° C.

creased with diameter, but the scatter of the values was greater than with C_M . The average value of G_M in the 120 to 139 μm group was 0.365 ± 0.018 mmho/cm² ($n=19$) whereas that in the 40 to 59 μm group was 0.213 ± 0.015 mmho/cm² ($n=9$). In a large fibre the tubules probably account for at least half the apparent membrane conductance (compare ref. 6) but the scatter of the results makes it difficult to assign a definite fraction to their contribution.

As have been pointed out by several authors⁷⁻⁹, the capacity can also be measured by determining the conduction velocity θ and the time constant τ_f of the exponentially rising foot of a propagated action potential. The equation for calculating the capacity is

$$C_f = \frac{DG_i}{4\theta^2 \tau_f} - G_f \tau_f \quad (1)$$

where D is the diameter, and C_f and G_f are the effective capacity and membrane conductance (in parallel) during the foot of the action potential. The second term on the right hand side is small compared with the first and can usually be neglected. At 21.5° C the average value of τ_f was 0.127 ms in twenty fibres with a mean diameter of 94 μm and a conduction velocity of 209 cm/s. The mean value of C_f derived from equation (1) was 2.56 $\mu\text{F}/\text{cm}^2$. As can be seen from the middle set of points in Fig. 2 the capacity obtained by this method is constant over the range of diameters from 56 to 141 μm .

To obtain "detubulated" fibres, bundles of muscle fibres were immersed in hypertonic Ringer fluid containing 400 mM glycerol for 1 h and were then returned to normal Ringer fluid for electrical measurements of the kind described here. The glycerol treatment either disrupts the tubules or disconnects them from the surface, and large molecules like ferritin or peroxidase can no longer enter the tubular system^{4,10-12}. Such fibres give no mechanical twitch but have fairly normal action potentials; their internal conductivity was found to be almost the same as in normal fibres.

The conduction velocity of fibres treated with glycerol was 60 per cent faster than normal, the average velocity in eleven fibres with a mean diameter of 90 μm being 341 cm/s; in these fibres the time constant of the foot of the action potential was 0.131 ms. The values of C_f calculated by equation (1), which are shown by the lowest set of points in Fig. 2, have a mean value of 0.9 $\mu\text{F}/\text{cm}^2$, in approximate agreement with the value of 1.2 $\mu\text{F}/\text{cm}^2$ obtained by extrapolating the low frequency capacity of normal fibres to zero diameter. The increase of conduction velocity is explained by the reduction of capacity associated with detubulation.

Experiments with rectangular pulses of current gave the low-frequency capacity of the same group of glycerol-treated fibres as 1.87 $\mu\text{F}/\text{cm}^2$ in fair agreement with Gage and Eisenberg⁴. One explanation of the difference between

high and low frequency capacities in these fibres is to attribute it to an imperfection in the membrane capacity¹³. Another is to assume that glycerol treatment does not, disrupt or disconnect the tubules completely.

The three curves in Fig. 2, which are a reasonable fit to the experimental points, were calculated from the theoretical model described by Adrian, Chandler and A. L. H.¹⁴. In this model, which was based on Falk and Fatt³ and Falk¹⁵, it is assumed that (i) the transverse tubular system is an open-mouthed regular network with a fractional volume (ρ) of 0.003 and a volume/surface ratio (ζ) of 10^{-6} cm (compare ref. 16); (ii) the conductivity of the luminal fluid (G_L) is 10^{-2} mho/cm⁻¹, that is, slightly less than the conductivity of Ringer fluid; (iii) tubular and surface membranes have identical properties, the capacity (C_W) being 1 $\mu\text{F}/\text{cm}^2$ and the conductance (G_W) 0.5×10^{-4} mho/cm²; these values were chosen to give an apparent membrane capacity and conductance similar to those found experimentally². In applying the theory to our results we used 0.9 $\mu\text{F}/\text{cm}^2$ instead of 1.0 $\mu\text{F}/\text{cm}^2$ for C_W ; the other constants were as proposed by Adrian, Chandler and A.L.H.

Curve 1 (low frequency capacity in normal fibres) is plotted from equation (22) of ref 14. Curve 2 (capacity from foot in normal fibres) is

$$C_f = \rho \sqrt{(\tau_f G_L C_W / 2\zeta)} \frac{I_1(a/\lambda_f)}{I_0(a/\lambda_f)} + C_W \quad (2)$$

where I_0 and I_1 are hyperbolic Bessel functions, a is the radius and λ_f is the space constant in the tubules of the exponentially rising voltage

$$\lambda_f = \sqrt{\left(\frac{G_L \zeta \tau_f}{2C_W} \right)} \quad (3)$$

Curve 3 (capacity from foot in glycerol-treated fibres) is

$$C_f = C_W = 0.9 \mu\text{F}/\text{cm}^2 \quad (4)$$

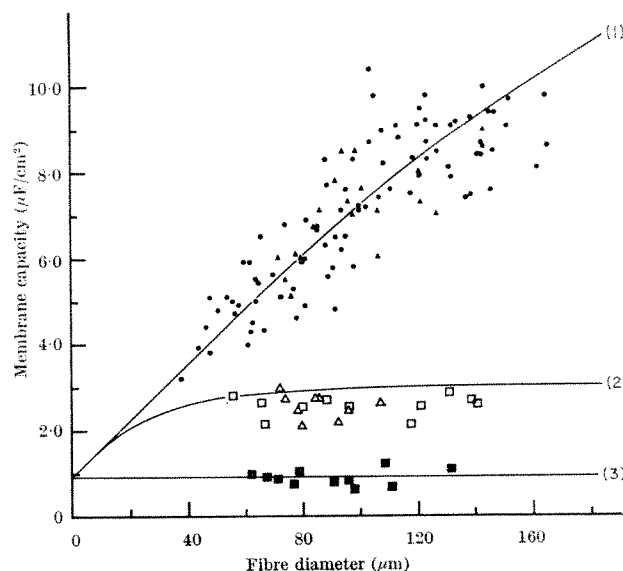


Fig. 2. Variation of capacity with diameter in twitch fibres. Curve 1, low frequency capacity in normal fibres. ●, Semitendinosus; ▲, sartorius. Experiments were carried out at 20° C. Curve 2, capacity during foot of action potential in normal fibres. □, Semitendinosus; △, sartorius. Experiments were carried out at 19.5° to 22.5° C. Curve 3, capacity during foot of action potential in detubulated fibres. Semitendinosus muscle was used at 20.0° to 21.8° C. The theoretical basis of the three curves is given in the text. The upper set of points includes the data from Fig. 1.

The physical basis of curves 1 and 2 is straightforward. From the values of ref. 14 it turns out that the space constant in the steady state is 100 μm , whereas the space constant for an exponentially rising voltage of time constant 0.13 ms is about 8 μm . Thus almost all the tubular network contributes to the low frequency capacity, but only the outer zone to the effective capacity during the foot of the action potential. The low frequency capacity therefore increases with diameter, but C_f is nearly constant for the range of diameters between 50 and 150 μm .

The general agreement between theory and experiment helps to validate the model of Adrian, Chandler and A. L. H., and provides strong evidence for attributing the high capacity of twitch fibres to the transverse tubular system. The estimates of tubular and surface capacity are in good agreement with those obtained from impedance measurements by Dr M. Schneider (personal communication).

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Effect of Fatty Acids on Contractility and Rhythm of the Heart

SINCE the finding in 1961 that free fatty acids inhibit the myocardial oxygenation of glucose¹, increasing emphasis has been placed on the role of free fatty acids as myocardial fuel². It is now known that free fatty acids inhibit glucose metabolism at several sites³. In studies on the human heart *in situ* free fatty acids are the dominant fuel in the fasting state⁴. It has therefore been suggested that free fatty acids are of major importance as a source of myocardial energy.

But circumstantial evidence has grown up to suggest that free fatty acids can in certain circumstances be toxic to the heart. In patients with acute myocardial infarction those with the most free fatty acid in the blood are most liable to develop arrhythmias^{5,6}. In dogs with ischaemic lesions of the myocardium, an abrupt increase of the plasma free fatty acids as a result of infusion of heparin and a lipid emulsion is associated with ventricular extrasystoles and ventricular arrhythmias⁷. These effects are on pathological hearts, but we have tested the direct effects of fatty acids on cardiac contractility in normal isolated hearts.

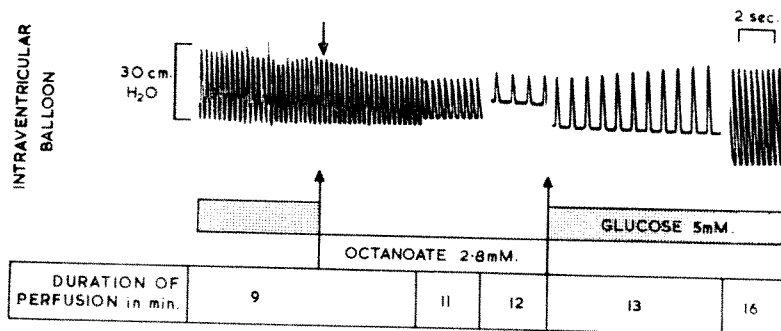


Fig. 1. Effect of octanoate on the contractility of isolated perfused rat heart. When the substrate of the isolated rat heart is changed (marked by arrow) from glucose to 2.8 mM octanoate contraction abruptly decreases and a high-grade atrioventricular conduction defect develops after 3 min. Normal contractions resume 3 min after changing back to a substrate of 5 mM glucose.

Isolated rat hearts were perfused with a Krebs-Henseleit bicarbonate medium, pH 7.4, with 5 mM glucose, or medium chain free fatty acid (octanoate) as substrate. Cardiac contractility was monitored by an intraventricular latex balloon⁸. Octanoate was prepared from octoic acid (British Drug House, Poole, Dorset) which was more than 97.5 per cent pure octoic acid; the rest consisted of other medium chain fatty acids as shown by gas-liquid chromatography. There were no known toxic substances in the octoic acid.

Table 1. EFFECT OF OCTANOATE ON CONTRACTILITY AND HEART RATE OF ISOLATED PERFUSED RAT HEART

Concentration of octanoate (mM)	No. of observations	Peak systolic pressure (per cent)	Heart rate (per cent)
2.800	(4)	59	81
1.400	(4)	83	98
0.700	(2)	62	100
0.350	(1)	70	100
0.175	(2)	69	100

Peak systolic pressure and heart rate are expressed as percentage of values obtained with 5 mM glucose as the only substrate. Mean values with glucose were: peak systolic pressure, 50 cm of H₂O; heart rate, 214 beats/min (fourteen observations in each case).

When the substrate of the isolated heart was abruptly changed from 5 mM glucose to 0.175–2.8 mM octanoate, within 1–2 min the peak systolic pressure fell to 59–83 per cent of the value with glucose as substrate (Table 1). With the highest octanoate concentration (2.8 mM) the effects were: (i) the heart rate decreased; (ii) a 3:1 atrioventricular block developed and the peak systolic pressure decreased to one-third after 2–3 min in two experiments (Fig. 1) and (iii) after re-exposure to glucose the rhythm reverted to normal in one experiment (Fig. 1), while in another experiment partial reversal to normal occurred. When the perfusion with 1.4 mM octanoate was prolonged, an irregular rhythm (quadrigeminy) developed after 5 min in one of seven experiments. Prolonged perfusion with 0.7 mM octanoate resulted in no rhythm disturbance.

In other experiments the rate of oxidation of 1.4 mM sodium octanoate-1-¹⁴C was 4.2 ± 0.8 (means \pm s.e.m., four hearts) $\mu\text{mole of octanoate/g wet weight/15 min}$, while 0.3 ± 0.06 μmole were recovered in the tissue lipid fraction. When 2.8 mM octanoate was added to 5 mM glycose-U-¹⁴C, the rate of oxidation of glucose was reduced to 11 per cent of the control value (four hearts). Although contractility was not measured, no serious arrhythmias developed.

These experiments show that octanoate, a medium chain length fatty acid could decrease the contractility of the isolated perfused rat heart and could cause arrhythmias. No similar changes occurred with albumin-bound long chain free fatty acid (palmitate) in physiological concentrations^{8,9} nor were rhythm disturbances noted when both glucose and octanoate were present in the perfusate. The metabolic behaviour of labelled octanoate was similar to that of labelled albumin-bound palmitate.

Octanoate also inhibited the uptake of glucose and pyruvate by the perfused rat heart^{10,11}. When hearts were perfused with both glucose and long chain fatty acid, the formation of triglyceride was increased¹², probably accompanied by a decrease in the tissue concentration of free fatty acid. It is not, however, known whether glucose could encourage the formation of triglyceride from octanoate.

It is attractive to postulate that the accumulation of free intracellular fatty acid is toxic to heart function, for example, by inhibiting oxidative phosphorylation¹³. This could explain (i) the effect of a very high concentration of fatty acid (2.8 mM octanoate) on the heart; (ii) the decreased efficiency of contraction of the isolated atrium when exposed to 2 mM hexanoate as sole substrate¹⁴ and (iii) the effect of very high circulating concentrations of long chain fatty acids on the rhythm of the infarcted heart, because uptake of free fatty acid by the ischaemic segment is proportional to the circulating concentration¹⁵, while oxidation of free fatty acids is probably reduced on account of hypoxia². Furthermore, since completion of this report, high but not low perfusate concentrations of a long-chain fatty acid, linoleate, have also been shown to be toxic to the perfused rat heart¹⁶.

The experiments reported here show that fatty acids in high concentrations can be toxic to the normal heart and can result in decreased contractility and rhythm disturbances.

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Electron Probe Microanalyser Localization of Lead in Kidney Tissue of Poisoned Rats

ATTEMPTS to explain the morphological changes of the kidney induced by lead poisoning have been concerned chiefly with nuclear and cytoplasmic inclusions^{1,2}. Cytoplasmic inclusions have been shown to contain Fe (ref. 1), but the composition of nuclear inclusions remains a subject for debate. It is still not clear whether lead is deposited within the kidney and some authors deny a direct action of this element in favour of a mechanism of indirect action through vascular changes³. Fortunately, the problem of detection of metallic localizations within the kidney is amenable to direct observations with the electron probe microanalyser, an instrument ideally suited to the purpose, with a detection limit of about 10^{-15} g and a spatial resolution of about $1 \mu\text{m}$ (ref. 4).

Kidney tissue was taken from Sprague-Dawley rats fed for 72 weeks on 1 per cent lead acetate solution⁵. Con-

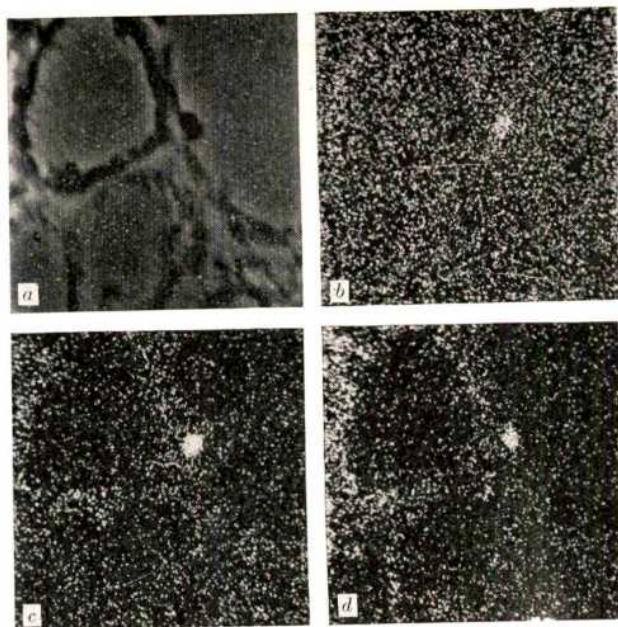


Fig. 1. Electron beam scans of rat kidney tissue, showing location of lead, calcium and phosphorus. Accelerating voltage 35 kV, specimen current 10^{-7} A. Each scan covers the same area of $100 \mu\text{m} \times 100 \mu\text{m}$. a, Specimen current; b, lead; c, calcium; d, phosphorus.

ventional paraffin embedded sections were made, mounted on pure silicon wafers, and the paraffin removed by xylene. Such sections, about $20 \mu\text{m}$ thick, were suitable for pathological purposes. Numerous glomeruli and normal and dilated tubules could be clearly defined under the microscope. In the electron probe (CAMECA), lead was readily found within cortical tubules in granules of differing shape and size. Fig. 1 shows a typical localization within a kidney tubule. Notable concentrations of calcium and phosphorus were found to be associated with the lead. Other elements were probably also present, but the chief constituents of the granules were lead, calcium and phosphorus. The scanning X-ray images (Fig. 1) also showed that calcium and phosphorus, in contrast to lead, appeared weakly throughout the tissue comprising the tubules.

These observations lend support to recent electron microscope work⁵ which suggested that lead poisoning is associated with lead-protein complexes. In that study lead was identified by a careful extraction process on many whole kidneys which isolated a quantity of inclusions sufficient for chemical analysis. Our work, in addition to localizing the lead, shows that calcium and phosphorus are co-deposited with the lead in these complexes, a result consistent with the hypothesis of the importance of lead-protein complexes.

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Direct-current Potentials in the Human Brain evoked during Timed Cognitive Performance

WALTER *et al.*¹ observed that when a human subject is presented with two sequentially paired stimuli so that the first serves to signal the occurrence of the second, a negative d.c. shift occurs during the interval between the stimuli in an averaged scalp EEG recording; he termed this phenomenon the "contingent negative variation" (CNV). CNV is frequently elicited with fixed foreperiod decision tasks, which may be said to include a "hold" phase, from the warning signal (WS) to the task stimulus (TS), and an "operate" phase, following the TS and terminating with the subject's response (R) to the TS (Fig. 1). CNV definitely occurs during the hold phase, so long as the subject is motivated and the task is sufficiently difficult²⁻⁶, but it has not been demonstrated that CNV is present during the operate phase. The tasks used in previous experiments have been so simple that TS and R occurred virtually simultaneously, and it could not be determined whether the termination of CNV covaries with the TS (the end of the hold phase) or with R (the end of the operate phase). In these experiments CNV was averaged during several complex cognitive tasks with considerably longer operate phases, and found to persist throughout the operate phase, for periods ranging up to 8 s after the TS.

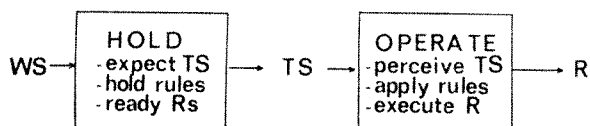


Fig. 1. Two phases of fixed-foreperiod decision tasks. The operate phase may be extended by making rules more complex.

Five male and five female students between 19 and 26 years of age served as subjects. The electroencephalogram and a record of eye movements were recorded from non-polarizable Ag-AgCl electrodes attached to the scalp at the vertex and over the left eye orbit, with a reference electrode on the left ear. These signals were amplified by means of low-level d.c. biological amplifiers set at a gain of 10,000, and averaged on a computer of average transients. In the initial experiment, the WS-TS interval (the hold phase) was set at 1 s. The reaction time of the subject was defined as the time from TS to R (the operate phase); R was recorded from a microswitch which reset a print-out frequency counter. The WS was a 50 ms 900 Hz tone pip, 80 db above absolute threshold. The TS consisted of two digits rear-projected onto a ground-glass screen set at eye level 70 cm in front of the subject, exposed for 100 ms at 27 foot-Lamberts. The digits subtended 4° of visual angle, and a fixation light was centred between them. Trials were presented in blocks of eighty. Inter-trial intervals were randomly ordered, and were 3-6 s long. At the beginning of every session the subjects were given the instructions for the task and then practised until their performance was error-free. Reaction times were examined periodically during practice; when 90 per cent of the responses were within 20 per cent of the mean reaction time, response variance was judged to be sufficiently low for CNV averaging. All CNV averages were computed from an N of 20, with sampling epochs of 2 s. To avoid eye-movement artefacts⁷ those averages were rejected in which more than 10 per cent of the sampled epochs showed any deflection in the eye-movement record. Two different sets of instructions for pressing the response button were used on different trials to manipulate reaction time. The first set, simple reaction time, required the subjects to press on every trial, to every TS. To ensure that they attended to

the digits and not to brightness changes alone, they had also to count the number of times during each session that the TS was changed. In the second set, selective reaction time, subjects were told to press only on certain trials and not on others—for instance, to press only on even numbers, or when the two digits were the same, or when the product of the digits exceeded a given number.

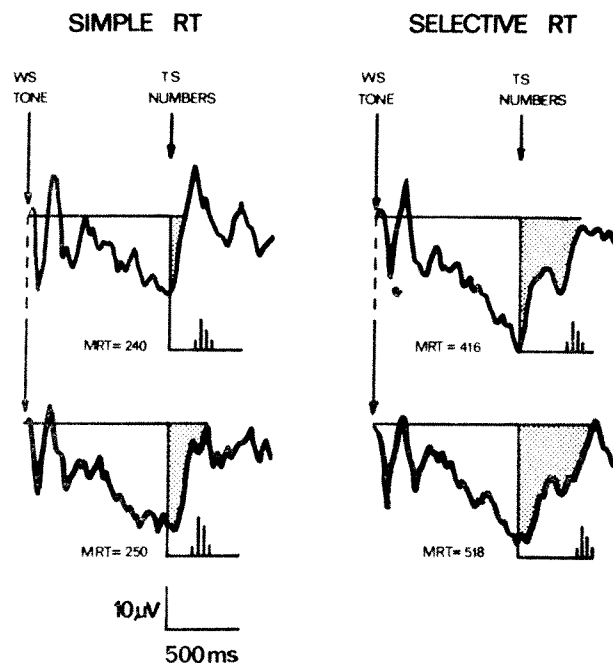


Fig. 2. Averaged contingent negative variations ($N=20$) for one subject showing the relation of corresponding reaction time frequency histograms to the amount of d.c. activity present after the TS (shaded areas). Relative negativity at the vertex is downward. Reference electrode: left earlobe. MRT, mean reaction time in ms.

CNV was elicited from all subjects. The time from TS to the termination of CNV (judged by its return to pre-trial voltage level) was highly correlated with reaction time: the Pearson product-moment correlation across all subjects was 0.81 (d.f. = 17; $P < 0.01$). In Fig. 2 averaged CNV records from one subject on two different sessions are juxtaposed with corresponding reaction time histograms for both simple and selective reaction time. The shaded areas under the curves indicate the amount of negative d.c. activity present between the TS and CNV termination. The amount of negativity is proportional to the reaction time, that is, to the length of the operate phase of the task. There was no significant correlation between CNV amplitude and time of CNV termination across tasks ($r=0.11$; d.f. = 17; $P < 0.10$); this rules out the interpretation that the correlation of CNV duration with reaction time is due to an amplitude-related change in the slope of the return of CNV to pre-trial baseline.

In the second experiment CNV was studied with two longer-lasting cognitive tasks, in which the operate phase could be extended out as far as 10 s. The first was a mental arithmetic problem. The WS-TS interval (the hold phase) was set at 3 s. The WS was a 50 ms 900 Hz tone pip, 80 db above threshold. The TS was a two-digit number spoken by the experimenter into an intercom on a timing signal. The subject was instructed to multiply the TS by a constant given to him before the first trial, and to state the product on hearing the instruction "report" delivered 4 s after the TS. The computations were sufficiently difficult that they required 3-4 s for solution. The operate phase of this task extended from TS to the verbal report, a period of 5-6 s, depending on the latency of the verbal response. The second task was a short-

term memory paradigm. No WS was presented because the longer duration of the stimuli made a foreperiod useless. The TS consisted either of a series of five digits presented acoustically at a rate of one digit per second, or a picture containing an array of conventional objects—houses, trees, animals and so on—projected onto a screen, 150 cm in front of the subject for 4 s. The task instructions were to remember the contents of the TS as accurately as possible, and then to recall them on the instruction "report" 6 s after the TS. The operate phase of this task began with TS offset, extending until the verbal response, a period of about 8 s. The 4 s stimulus exposure period possibly contained elements of both hold and operate functions. As in the reaction time sessions, CNV averages were computed from an N of 20. Sampling epochs were 16 s in duration, in order to include WS, TS, R, and the termination of CNV. Eye-movement artefacts were eliminated in the same manner as in the reaction time sessions.

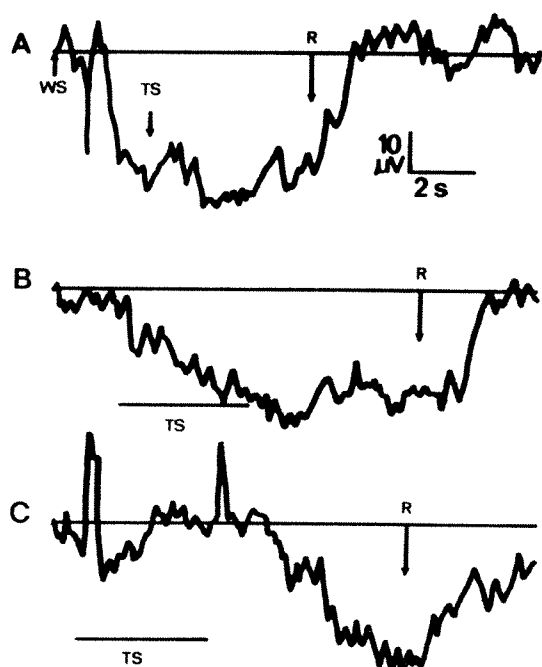


Fig. 3. Averaged contingent negative variations ($N=20$) for three subjects showing the relation of performance of different cognitive operations to duration of contingent negative variation. Relative negativity at the vertex is downward. Reference electrode: left earlobe. A, Multiplication task; B, digit storage and recall; C, storage and recall of pictorial stimuli.

In the mental arithmetic task a reliable CNV developed during the WS-TS interval (Fig. 3A) similar in amplitude and slope to the negativities previously observed during the "hold" phase. During the TS-R interval, or operate phase, CNV was sustained, peaking an average of 1.25 s after TS. Thus, if anything, the operate phase of the task increased the amplitude of the CNV that had developed in the foreperiod. Although the time of CNV termination varied considerably between subjects, in all subjects it occurred after the onset of the verbal response. In the short-term memory tasks CNV was present during the operate phase of both digit and pictorial recall. It can be seen in Fig. 3B that on the digit recall task, CNV developed during the TS presentation reaching peak amplitude shortly after the final digit in the series, and continuing until the verbal report some 10 s later. On the picture recall task (Fig. 3C) CNV developed briefly after the onset of the TS, lasting for only about 2 s, resuming an average of 1.5 s after the offset of the TS and continuing until verbal report. Two prominent positive waves occurred in the

latter records, time-locked to the onset and offset of the stimulus, possibly related to the on and off responses described by Clynes⁸. The picture recall task did not evoke CNV as reliably as any of the other tasks. CNV, occurred on 56 per cent of the sessions while flat averages, showing no d.c. shifts, were observed in the remaining 44 per cent.

There are three current hypotheses about the functional significance of CNV. The first claims that CNV is the human equivalent of the negative d.c. shifts described in cats by Rowland^{9,10}, and simply another correlate of general behavioural activation. Although compatible with the results of this specific study, this theory is improbable for CNV cannot be elicited in man by one of the most commonly accepted activation paradigms, the orienting response to sensory stimulation, and indeed the human orienting response seems to produce a positive, rather than a negative, deflexion in the averaged EEG (ref. 11). The second hypothesis holds that CNV is a "readiness" potential constituting part of the evoked motor responses which accompany motor movements^{12,13}. This interpretation is weak because CNV has been observed in subjects who are producing no overt motor movements but simply anticipating an interesting stimulus¹⁴. Furthermore, in this study CNV lasted for periods of up to 8,000 ms after the TS, often with a peak 4,000 ms before the command eliciting the motor response; whereas negativities preceding previously described motor potentials last only 300–800 ms, peaking immediately before the overt movement. The third hypothesis suggests that CNV is related to expectancy and attention to stimuli¹⁵; but while these terms apply to the empty-interval hold phase, it is not clear how they apply to the complex operate phases used here. In view of this, it is simpler to forgo these hypotheses and hold that CNV occurs during any period of intense, short-term concentration, and not only during simple anticipatory states, which constitute one of several ways of inducing concentration. Thus the behavioural correlates of CNV may indicate the presence of a functional system distinct from the general arousal system which serves to facilitate concentration on all phases of task performance, perhaps by minimizing distractions or stabilizing central and peripheral posture.

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Energy Flow and Species Diversity in a Marine Phytoplankton Bloom

MARGALEF¹ has attempted to predict the ways in which certain descriptive (but quantitative) properties of an ecosystem will change during the development of the ecosystem, or when the system is subjected to disturbance. Briefly, the hypothesis he uses states that the transition of an undisturbed ecosystem from a juvenile to a more mature condition is characterized by changes in the system such as the following: an increase in the amount of primary production; an increase in the total biomass; a decrease in the ratio of primary production to biomass; a greater efficiency of production; an increase in the species diversity; a decrease in the ratio of production to respiration; an increase in the importance of stratification and spatial organization. Some of these ideas have been criticized by Slobodkin² as lacking in empirical justification. The results of our field observations on the energy flow in a marine phytoplankton population³ lend support to the views of Margalef and of Odum⁴, but certain weaknesses in the hypothesis are exposed when an attempt is made to apply it to real data.

The observations were made during the spring diatom bloom in St Margaret's Bay, Nova Scotia; all measurements were made at the same station, roughly one mile from the shore, depth 58 m. Six depths were sampled: 1, 5, 10, 15, 25 and 40 m. The station was occupied eleven times between March 28 and April 29, 1969; all phases of the spring maximum were covered. The biomass of plankton was estimated, as particulate carbon, as cell density, and as chlorophyll *a*; relative production was expressed in terms of the quantity $k_b = P(z)/I(z)$ where $P(z)$ is the primary production at depth z measured by the *in situ* ¹⁴C technique and $I(z)$ is the available light at that depth; both P and I are in calories⁵. The ratio of photosynthesis to respiration (P/R) was estimated by the dissolved oxygen technique. Species diversity D was computed from the equation $D = -\sum p_i \log_2 p_i$ where p_i is the probability that an individual belongs to the i^{th} species; the pigment diversity, c/a , was measured as the ratio of chlorophyll *c* to chlorophyll *a*. The age of the bloom was assessed by taking the arbitrary zero of time on the first sampling day.

The partial correlation matrix for the results is shown in Table 1. The ratio of chlorophyll *c* to chlorophyll *a* was well correlated with species diversity. In this respect the c/a ratio is analogous to the pigment index (that is, the ratio of optical densities at 430 nm and 665 nm of acetone extract of plankton) of Margalef, which has been found to be a good predictor of species diversity. Both species diversity and c/a had significant negative correlation coefficients with the age of the bloom. Neither species diversity nor c/a was significantly correlated with the concentration of either phosphate or nitrate, but in every case the sign of the correlation coefficient was as expected (negative). Low values of species diversity were associated with high values of P/R ratio. There was a significant trend of decreasing P/R with age of the bloom. The relative productive efficiency k_b increased with depth. There was a strong negative correlation between k_b and species diversity.

These results are entirely consistent with current ideas of ecosystem dynamics as set out by Margalef and Odum. One might also have anticipated significant correlations between species diversity and depth, P/R and nutrients, and k_b and age, none of which materialized.

At first sight, however, the highly significant negative correlations between species diversity and all measures of the standing stock of phytoplankton seem to run counter to Margalef's general thesis that increases in biomass are associated with increases in diversity. But we feel that the contradiction might be resolved by drawing a distinction between short term and long term responses of the system. In our opinion, the confusion of many different time scales constitutes an important weakness in present ecological theory. If these concepts are to be made operational—and they must if they are to be relevant—then some basis must be found to disentangle the confounding of processes with widely divergent time constants. We must know whether we are to apply the same principles to systems with a lifetime of the order of days as to systems more appropriately considered on the geological time scale. This point has been made previously by Deevey⁶.

Table 2. PARTIAL CORRELATION COEFFICIENTS BETWEEN VARIOUS MEASURES OF P/B AND SOME OBSERVED QUANTITIES

Each coefficient is based on 34 degrees of freedom

	$k_b/\text{chl}a$	$k_b/\text{cell numbers}$	k_b/carbon
Depth	0.71*	0.53*	0.52*
PO_4	0.49*	0.31	0.40†
NO_3	0.39†	0.19	0.28
c/a	-0.05	0.00	-0.11
Spp. diversity	-0.08	0.11	-0.46*
P/R	-0.05	-0.09	0.19

* Exceeds value expected at 1 per cent probability level.

† Exceeds value expected at 5 per cent probability level, but less than 1 per cent.

Margalef considers the ratio P/B (primary production to total biomass) to be an important parameter in ecosystem theory. In our data the ratios of k_b to any of the measures of standing stock give estimates of P/B . The quantity of carbon should be the best measure of total (that is, both living and dead) biomass, for in counting cell numbers one normally excludes "dead" cells, and chlorophyll *a* can be measured free from its degradation products. The ratio k_b/carbon , therefore, is closest to the intent of the parameter P/B . It is interesting then, that k_b/carbon was the only measure of P/B which had a significant partial correlation with species diversity (Table 2). This not only supports the principle that increasing species diversity is associated with a decrease in P/B , but also bears out the contention of both Margalef and Odum that non-living biomass may be as important as living material for the organization of the ecosystem. This observation is not without relevance to the controversial status of "organic detritus" in the sea.

The following argument could, however, be made: for some ecosystems, such as forests, where all parts of the cycling system are readily accessible to study, B should include both living and non-living biomass. But in studies of ecosystems such as the plankton ecosystem where an important part of the materials cycle (the benthos) is ignored, B should more properly be a measure of productive potential. In this case P/B would be expressed in terms of $k_b/\text{chlorophyll } a$. This ratio gave the

Table 1. PARTIAL CORRELATION COEFFICIENTS AMONG THE VARIOUS QUANTITIES MEASURED IN THE STUDY

Each coefficient is based on 34 degrees of freedom

	Depth	PO_4	NO_3	$\text{Chl}a$	c/a	Cell number	Carbon	Spp. diversity	P/R	Ag
PO_4	0.76*									
NO_3	0.74*	0.90*								
$\text{Chl}a$	0.02	0.04	-0.09							
c/a	-0.05	-0.30	-0.22	-0.03						
Cell number	0.05	-0.01	-0.08	0.81*	0.19					
Carbon	-0.20	-0.20	-0.27	0.61*	0.20	0.45*				
Spp. diversity	-0.12	-0.31	-0.18	-0.72*	0.32†	-0.44*	-0.48*			
P/R	-0.21	-0.03	-0.04	0.36†	-0.16	0.08	0.30	-0.40†		
Age	0.00	-0.36†	-0.42*	-0.14	0.33†	0.01	0.05	0.42*	-0.38†	
k_b	0.31†	0.24	0.03	0.80*	-0.08	0.50*	0.43*	-0.61*	0.29	-0.02

* Exceeds value expected at 1 per cent probability level.

† Exceeds value expected at 5 per cent probability level, but less than 1 per cent.

Table 3. SUMMARY OF STEPWISE MULTIPLE LINEAR REGRESSION ANALYSIS.

	Regression coefficient	s.e. of regression coeff	R	ΔR^2
Chla	0.00325	0.00048	0.800	0.6392
Depth	0.00079	0.00023	0.853	0.0889
Cell number	-0.00671	0.00176	0.896	0.0747
NO ₃	-0.00477	0.00165	0.918	0.0398
PO ₄	0.02935	0.01412	0.926	0.0141
Spp. diversity	0.00156	0.00293	0.928	0.0034
c/a	0.00225	0.00358	0.928	0.0019
Carbon	-0.00001	0.00001	0.929	0.0012
P/R	0.00020	0.00072	0.929	0.0005
Age	0.00006	0.00018	0.929	0.0004
Constant term	-0.1975			

Analysis of Variance			
	d.f.	Sum of squares	F ratio
Regression	10	0.009	21.45
Residual	34	0.001	

Dependent variable is k_b . Quantity R is the multiple correlation coefficient. ΔR^2 is the increase in the amount of the variance of k_b which is explained by including a particular variable in the regression. The standard error of the estimate is 0.0065.

best correlation with inorganic nutrients, but no correlation with species diversity (Table 2). The difference between this and the previous interpretation is in the amount of emphasis placed on the system itself compared with the importance attached to the organisms and their interactions.

If the only object had been to establish a predictive equation for primary production, it seems that much less work could have been done at the sacrifice of only a little information. Table 3 summarizes the stepwise regression calculation. The square of the multiple correlation coefficient is the fraction of the variance in k_b explained by the regression. Concentration of chlorophyll a could account for 64 per cent of the variation in k_b . Depth accounted for a further 9 per cent of the variance, cell number 7 per cent and nutrients 4 per cent. The other six quantities measured accounted for only a further 2 per cent of the variation in k_b . This is not to deny the importance of these six quantities; the point is that they made little further refinement to prediction of k_b , once changes in chlorophyll, depth, cell number and nutrients had been taken into account. This supports the argument of Platt⁵ that changes in the chlorophyll content of algal cells in response to nutrients, temperature and other environmental factors enhance rather than detract from the value of chlorophyll as a standing stock index in the prediction of primary production.

We have thus found some empirical justification, in natural conditions, for Margalef's hypothesis of ecosystem dynamics. The chief difficulty we find in trying to apply the ideas to real situations is a lack of clarity concerning the various time scales involved; for example, values of P/B could be quite different, depending on the times over which P and B are averaged. The main weaknesses we find in our own treatment are, first, that the inadequacies of the linear model may have obscured some important information and, second, that we have ignored Margalef's timely hint of the importance that derivatives (in the sense with which the word is used in calculus) must play in the future progress of ecological understanding.

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¹ Margalef, R., *Perspectives in Ecological Theory* (Univ. Chicago Press, Chicago, 1968).

² Slobodkin, L. B., *Science*, **164**, 817 (1969).

³ Platt, T., and Subba Rao, D. V., *J. Fish Res. Board Canada* (in the press).

⁴ Odum, E. P., *Science*, **164**, 262 (1969).

⁵ Platt, T., *Limnol. Oceanogr.*, **14**, 653 (1969).

⁶ Deevey, E. S., *Limnol. Oceanogr.*, **14**, 313 (1969).

Distribution Pattern of the Duplication of Discoveries

PRICE¹ conjectured that a Poisson distribution might be a good fit for the pattern of incidence of multiple discoveries. We have fitted a modified Poisson distribution to the frequency of duplication of discovery of antibiotics.

We have considered the discovery of an antibiotic to have been duplication if it was identified with another antibiotic and so reported in published documents, irrespective of whether the compounds were produced by one and the same or different species of microorganisms². We used the data reported earlier³ on the frequency and duplication of discovery of antibiotics during 1907-1966 after correcting a few minor discrepancies. As there was practically no duplication of discovery of antibiotics from bacteria, we have dealt only with the pattern relating to two groups: (1) fungi, algae and lichens, and (2) actinomycetales.

A normal Poisson distribution did not fit the distribution pattern of duplications, but a modified version³ did. For such a distribution, the p.d.f. is

$$\left(\frac{c}{c+1}\right)^p \frac{p(p+1)(p+2)\dots(p+(r-1))!}{(r-1)!(r+1)^r} \quad (1)$$

where p and c are constants to be estimated using the following relations

$$\text{mean of } X = p/c \quad (2)$$

$$\text{var } (X) = p/c + p/c^2 \quad (3)$$

Using equations (2) and (3), p and c were estimated separately from the data on antibiotics derived from the two groups of organisms and for the pooled data. Using formula (1), the expected frequencies were computed. A χ^2 test was done. To fit the distribution, the upper tail of the distribution from duplication frequency 8 and above was omitted. Table 1 gives details of the goodness of fit. In all three cases, the goodness of fit was confirmed.

Table 1. OBSERVED AND EXPECTED FREQUENCIES OF DUPLICATION, AND TEST OF GOODNESS OF FIT

No. of times duplicated	No. of antibiotics from fungi, algae and lichens		No. of antibiotics from Actinomycetales		Total	
	Observed	Expected*	Observed	Expected*	Observed	Expected
0	371	364.2	1,018	1,011.6	1,380	1,380.5
1	39	44.3	79	81.9	118	121.5
2	12	15.8	20	20.5	32	44.2
3	7	6.9	15	13.3	22	20.2
4	2†	3.3	7	6.7	9	10.1
5	4†	1.6	7†	3.5	11	5.4
6	2†	0.8	4†	1.9	6†	2.9
7	1†	0.4	1†	1.1	2†	1.7
	2.98		8.23		12.25	
χ^2_{cal}	9.49 for 4 df		12.59 for 4 df		12.59 for 6 df	

* Value corrected to first decimal place.

† Grouped data used.

There thus seems to be predictable regularity in duplications, and Price's conjecture about the pattern of distribution of multiple discoveries is confirmed for antibiotics, even in cases where there are no duplications.

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¹ Price, D. De S., *Little Science, Big Science*, 66 (Columbia University Press, New York, 1963).

² Neelameghan, A., *Library Science with a Slant to Documentation*, **5**, 264 (1968).

³ Kendall, M. G., and Stuart, A., *Advanced Theory of Statistics*, second ed., 1 (Hafner, New York, 1958).

Book Reviews

HISTORY OF BIOCHEMISTRY

The Chemistry of Life

Eight Lectures on the History of Biochemistry. Edited by Joseph Needham. Pp. xxx+214. (Cambridge University: London, April 1970.) 60s; \$9.50.

HISTORICAL aspects of science nowadays tend to occupy little time in the education of scientists. Students are liable to argue that there is so much to be learned about the present state of their subject that they cannot spare time for the study of the history. The truth is that some time spent on historical aspects is by no means wasted because a full understanding of scientific knowledge and of the nature of research requires a study of the historical development of the subject. In the introduction to this book the editor mentions that Frederick Gowland Hopkins, the "father" of British biochemistry, never lost an opportunity of referring to events of historical interest. "Whether it was van Helmont capturing the 'wild-gas' in breweries, or Captain Lind protecting his men from scurvy with citrus fruits, or Berzelius finding lactic acid in the muscles of hunted stags in 1808, or Fick and Wislicenus romantically climbing the Faulhorn to settle the relation of protein metabolism to muscular work—all were brought in to give colour and perspective, to enlarge our understanding of our past."

This book is the outcome of a course of lectures given by Cambridge biochemists some ten years ago. Joseph Needham, in his introduction, deals with the forerunners of biochemistry beginning with the Greek pre-Socratic philosophers of the sixth and fifth centuries BC, early Indian and, of course, Chinese achievements through the middle ages until the eighteenth century. His valuable list of references leads those interested to the sources. Robert Hill describes the growth of our knowledge of photosynthesis and Malcolm Dixon discusses the history of enzymes generally and of biological oxidations in particular. E. F. Gale covers the development of microbiology from the days of Leeuwenhoek to the twentieth century, discussing the argument on spontaneous generation, the nature of fermentation and the germ theory of disease. A long and highly original section, occupying almost one third of the book, written by Kendal Dixon, is headed "Some Biochemical Signposts in the Progress of Neurology". The concepts of Vesalius, Descartes, Willis and Albrecht von Haller on the function of the brain, as well as the eighteenth and nineteenth century ideas and experiments, are discussed and documented by detailed quotations. F. G. Young deals with the evolution of the concept of animal hormones and Leslie Harris with the discovery of vitamins. Mikuláš Teich traces the foundations of modern biochemistry, beginning with Lavoisier. The final chapter, by Sir Rudolph Peters, entitled "Some Lone Pioneers of Biochemistry in the 19th Century", selects for treatment Wollaston (who discovered uric acid in the chalky deposits of gout, and cystine in a kidney stone), Andrew Buchanan (who did important work on blood coagulation), Bence-Jones (whose name is still well known as the discoverer of a special urinary protein), Alfred Garrod (an expert on gout)

and his son Archibald Garrod (the originator of the concept of inborn errors of metabolism).

Reading this book brings home the difficulties which future historians may face when they attempt to write accounts of the more recent events in science. The rapid growth and the ever increasing complexity of science makes the task of the historian a very formidable one. So I think that it is becoming increasingly important that those who have personally participated in the progress of science, or witnessed it from close quarters, should be encouraged to write histories of their own special field (as Keilin did in his *History of Cell Respiration and Cytochrome*). Others are unlikely to master adequately the intimate details. As Watson's *Double Helix* forcefully illustrates, the published papers often omit historically interesting and relevant aspects of scientific developments. Already long ago Ampère was blamed that "having built up a perfect demonstration he removed all the traces of the scaffolding by which he had raised it". In these days of team work, and of frequent personal contacts between scientists, ideas tend to mature informally and the published accounts report too little of the maturing process. It is therefore welcome news that there are now historians of science who concern themselves with very recent history and try to supplement information contained in ordinary research papers by personal interviews with the scientists who have made history.

H. A. KREBS

HOME CURES

American Indian Medicine

By Virgil J. Vogel. Pp. xx+583. (University of Oklahoma: Norman, February 1970. Distributed in the UK by Bailey Bros. and Swinfen.) 118s; \$12.50.

THE title of the book might suggest that the author was a medical specialist or at least a physical anthropologist, and it is a pleasant surprise to find that he is, in fact, a Chicago historian. Dr Vogel, by this important reference work, demonstrates clearly that historians can contribute no less positively to medical anthropology.

The principal subject matter of the book is concerned with the influence of American Indian medical practices on European settlers. As the preface points out, the majority of studies on Amerindian medicine concentrate on the ritual aspects and tend to neglect the extensive use of more rational therapy. In particular, the Amerindian has investigated and selected for use a surprisingly large number of indigenous plants.

The case for the influence of Amerindian herbs and drugs in the western world was in some quarters resisted, perhaps because substances such as decomposed corpse and menstrual blood were also part of their materia medica. But the sixteenth century European pharmacist was no better, with his mummy dust, pigeon excrement and stag's penis. The fact is that, as regards Amerindian plant lore, we have a very interesting example of counter-acculturation, with the so-called dominant progressive group receiving varied information of lasting use and value (cocaine, cochineal, cinchona, curare, ipecac, vanilla, guaiacum, chenopodium, balsams and so on). But Vogel is not trying to argue that the American Indians generally used scientific methods of experiment and reasoning in adopting remedies and methods of treatment, although the treatment of fractures, dislocations, wounds and bites were usually rational and often effective. He simply wishes to provide detailed evidence to show that combined with the superstitions and untested beliefs which were so much a part of their medicine, there was also a hard-core of procedures and discoveries which have influenced, or at least put into better perspective, the development of medicine in the western world. For instance, it is clearly wrong to give the British credit for a cure for scurvy, when it is known that the Indians

successfully treated Jacques Cartier's men for the disease on the St Lawrence River as early as 1535. Even the shamanistic practices are clearly worth considering, as Vogel has done, for more than their ritualistic relevance. As Professor Ackerknecht pointed out some years ago, these apparently useless rites may in actual fact have been of considerable psychotherapeutic value, and should not be underrated in this respect.

In terms of overall content, the first third of the book considers Indian theories of disease and shamanistic practices, a regional survey of the early observations on Indian medicine made by European settlers, and of the influence of such medicine in the early colonies. Following this is a short but useful account of the historical evidence for Amerindian health during the earlier period of European contact. The chapter on Indian therapeutic methods is long and detailed, and together with the accompanying appendix on American contributions to pharmacology, this latter part of the book will remain an important reference source for many years. The bibliography of forty-four pages is thorough and up to date.

DON BROTHWELL

IN SEARCH OF A ROLE

Training Tomorrow's Psychiatrist

The Crisis in Curriculum. Edited by Theodore Lidz and Marshall Edelson. Pp. xiv + 94. (Yale University: New Haven and London, June 1970.) 18s paper; 60s boards.

DURING the ten years since the Mental Health Act (1959) became law, there has been a constant pressure for change in the practice of psychiatry, not only in Britain but throughout the western world. The pressure comes partly from within the profession itself: in many European countries, psychiatry has been dominated, to its detriment, by the vested interests of neurologists. In turn, Holland, France and West Germany have taken steps to separate these two related and yet profoundly different disciplines. In Britain, the Royal Medico-Psychological Association has been in labour for the past six years trying to give birth to a Royal College of Psychiatrists. In America, the 1963 "Community Mental Health Centers Act" has opened up new professional careers, challenging the dominance of private-practice individual psychotherapy.

To an even greater extent, however, the pressure for change has come from the public, in the form of increased readiness to seek psychiatric help, and raised expectations of the help which should be available to relieve emotional distress. Public demands and political initiatives have accelerated changes in the patterns of mental health care on both sides of the Atlantic. In Britain, the recommendations of the Todd Commission, which advocated that more attention be given to the behavioural sciences, to psychiatry and to community medicine in the future training of doctors, have nowhere yet been implemented; but significantly, the Seeborn Report on the Personal Social Services, published shortly after Todd, has already transformed and greatly extended the role of the social work profession. (In a little-regarded paragraph, the Seeborn Committee drew attention to the apparent confusion among psychiatrists as to their proper role in dealing with the enormous problem of minor mental ill-health among children and adults.) In the United States, the very multiplicity of experiments in trying out new methods of providing mental health care has added to the confusion: psychiatrists, psychologists, social workers and non-professional community leaders have all severally claimed the right to direct and control community mental health programmes.

It was against this background of tumult and confusion that a group of psychiatrists met recently at Yale to discuss the future training of psychiatrists. Four of their papers are presented in this booklet, together with

a foreword by Dr Theodore Lidz. The two opening papers stress the need for psychiatrists to have medical training, but suggest that the biological side of that training can be attenuated in favour of early specialization in psychiatry proper. The third paper outlines an ambitious and clearly intellectually demanding undergraduate elective course in behavioural science for future psychiatrists, while the fourth examines the conflicting views about what constitutes "community psychiatry", and presents some guarded opinions about how psychiatrists should be trained for this still ill-defined field of action.

Dr Lidz's preface ends with the following sentences: "The papers included in this volume do not provide solutions nor do they cover all of the significant problems confronting psychiatric educators. They consider some of the significant issues and raise many questions. In a sense, the meeting at which they were given explored the need for new directions in educating psychiatrists, and indicated the need for a conference at which the issues could be examined more fully and definitively". These sentiments, echoing the aside in the Seeborn Report, are no less true of British than of American psychiatry today.

G. M. CARSTAIRS

SLAVE SYNTHESIS

The Atlantic Slave Trade

A Census. By Philip D. Curtin. Pp. xix + 338. (University of Wisconsin: Madison and London, May 1970.) 71s; \$7.50.

IN the preface to this book, the author stresses that it is neither a textbook nor a definitive monograph. In common with most other academics Africanists have contributed, during the past quarter of a century, an increasing number of works at both ends of the publishing spectrum. Young scholars have been more or less forced to undertake their first major investigation in the field and on relatively narrow topics and, in consequence, the library thesis (or synthesis) has become less and less acceptable. Experienced scholars have been tempted by publishers to write the broad works of synthesis for which there would seem to be an ever increasing demand. Conversely, book-length syntheses of fairly large topics at an intermediate level have been relatively rare. The Atlantic slave trade was just such a topic and no real attempt had been made to review the considerable secondary literature on it, most of which had been published since 1945. In attempting to do so, Professor Curtin conceived his task as "building with the bricks that exist, not in making new ones". Where there is a consensus among authorities he has let it stand; where no consensus exists, he has looked further but stopped short of original research.

As the subtitle indicates, this book is a census. Its aim is to appraise and collate information on the African origins and New World destinations of the slaves, the numbers involved in the transatlantic trade and the fluctuations therein through almost four centuries. It reflects a recent trend towards quantification in historical scholarship. There are several semi-logarithmic graphs, eighty-three tables and an acknowledgement in the preface to the assistance of the University of Wisconsin's Computing Center. While the author stresses that the estimates may be in error by as much as twenty per cent, he nevertheless demonstrates their value in measuring the relative importance of different geographical branches of the trade at different times. For example, less than a twentieth of all the slaves were imported into British North America, whereas more than a third went to Brazil. Contrasts were almost as marked through time, so that possible large errors do not seriously mask the trends.

The book is well presented, contains numerous simple but effective maps, and is well indexed. It can be read or

referred to with equal facility. Apart from a final interesting chapter on mortality it sticks closely to the stated theme and adheres to its self imposed rules.

One hopes that in the nineteen-seventies more scholars in more fields will publish book-length syntheses of this type. If it does not happen, then even the best and most significant findings of original scholarship will fail to get into the textbooks. The gap between research and teaching will then have grown too wide to be bridged.

G. MALCOLM LEWIS

ONE MAN'S CONTRIBUTION

The Transmission of Passive Immunity from Mother to Young

By F. W. Rogers Brambell. (North-Holland Research Monographs—Frontiers of Biology, Vol. 18.) Pp. xvi+385. (North-Holland: Amsterdam and London, 1970.) Hfl. 65; 152s; \$18.20.

WITH the death of Professor Brambell developmental biology has lost one of its finest and most respected mentors. The publication of this volume at such a sad time was coincidental but perhaps appropriate. In it, his most important contribution to our knowledge of the acquisition of humoral immunity by the foetus and newborn is extensively described with characteristic thoroughness and lucidity. Published as one in a series of excellent monographs it presents a systematic examination of both prenatal and postnatal transmission of maternal antibodies to the young in a variety of species.

The writing of a monograph has unfortunately become one more hurdle in a scientist's career, something that one is expected to do. As a result the average reader is bombarded by expensive prestige books often of the very lowest intellectual quality, shrapnel in the information explosion. Professor Brambell's book is most certainly not one of them. It is simply an excellent review and summary of one man's contribution to his subject. The quality of the book lies in the very high standard of experimentation presented, in the clear exposition of theory, and in the intellect of the author.

For each species there is a detailed account of placentation and the structure of the foetal membranes, followed by an elegant analysis of the mode of transfer of antibodies both before and after birth. The species examined include birds, marsupials, rodents, the cat, the dog, the hedgehog, ruminants, the pig and, finally, monkeys and man. One splendid chapter considers the association between the transmission of antibodies and haemolytic disease of the newborn in many species and ends with an intriguing paradox; the absence of haemolytic disease in newborn ruminants. Experimental immunization studies show the development of the appropriate antibodies and their subsequent transmission to the young through the colostrum. Such antibodies are haemolytic *in vitro* and yet the young are unaffected.

The final section is an erudite collection of conclusions and hypotheses. With its emphasis on the selective transmission of antibody and the probable presence of specific globulin receptor sites on cells it clearly outlines the resemblances between antibody transmission and anaphylactic sensitization. How can a complex protein like antibody globulin survive its passage through a cell packed with proteolytic enzymes? Professor Brambell argues with conviction that specific receptors bind to the globulin and inhibit enzyme digestion within the phagosomes. Saturation of these receptors causes excess globulins to be degraded; a simple yet elegant theory amply supported by experimental evidence. Indeed, most statements made in this book are backed by abundant evidence. Forty-two pages of references may seem excessive in some books. In this one they are an integral and vital part.

The price of this volume will no doubt limit it to the

shelves of institute libraries and a few well-heeled developmental immunologists. It is no doubt a book for the specialist in spite of its obvious relevance to practical clinical problems. To one reviewer at least it is a source of delight and an excellent reminder of the qualities of the late Professor Brambell.

G. A. CURRIE

LIVELY EMBRYOLOGY

Cells Into Organs

The Forces that shape the Embryo. By J. P. Trinkaus. (Foundations of Developmental Biology Series.) Pp. xvi+237. (Prentice-Hall: New Jersey and Hemel Hempstead, 1969.) n.p.

EARLY students of cell behaviour in morphogenesis were, of course, well aware of the possible roles of change in cell size and cell shape. But they undervalued cell movements and were often over impressed by the importance of differential activity. We owe to Wilson, Vogt, Pasteels and Holtfreter the first development of a fresh approach the outcome of which is very well surveyed in Dr Trinkaus's book. It is noteworthy that he hardly has occasion to mention cell division and that when he does it is without exaggerated respect. Nevertheless, it is fair to suspect that it will return to a qualified favour in the future—not as solely responsible for any morphogenetic change, but as an important participant in some.

At the present time the parameters of cell behaviour most intensively studied are the strength and specificity of inter-cell adhesions, the modes of cell motility, and the social behaviour of cells the properties of which may depend critically on the company they keep. In each case the part of the cell most immediately involved is its surface. In each case a combination of *in vivo* and *in vitro* work on living cells must be supplemented by fine structural study and chemical analysis. Already some seemingly complex pieces of morphogenetic activity promise to yield to analysis in fairly simple terms.

Trinkaus provides a good, and much needed, account of this lively field. It will serve equally as a primer of cell biology for embryologists and of morphogenesis for cell biologists. He draws on a very wide range of material and is helpful in making clear the nature of contemporary disagreements. He is, however, more than clear; he succeeds in conveying real enthusiasm for the problems he describes.

D. R. NEWTH

PICTORIAL ULTRASTRUCTURE

Cells and Tissues by Light and Electron Microscopy

Vol. 1: Microscopie Photonique et Electronique de Tissus et de Cellules. English version by Edmund B. Sandborn, French by R. Gagnon, P. Jean and B. Messier. Pp. xiii+366. (Academic: New York and London, March 1970.) 135s.

ATLASES of ultrastructure are now commonplace and any new contribution has to offer something special to compete. This work is larger than most; it is, in fact, the first of two volumes and deals with the cell and basic tissues such as epithelia, muscle, nerve, connective tissue and lymphoid organs. Volume two will cover other systems such as endocrines, circulation, digestion, excretion, and the like. The special feature one is led to expect from the publisher's leaflets and from the dust cover is a thorough correlation of light and electron microscopy. It is claimed that the book "is a coordinated study of animal cells and tissues as seen in the light and electron microscope. . . . It is the first attempt at providing a study of biological structure which includes both light and electron microscopy". From this one might expect a careful comparison between the histology and histochemistry of a tissue and its ultrastructure. This is something that is lacking in the literature and it would be extremely valuable. This

book makes no attempt to cover the gap. To begin with, fewer than one in seven of the photographs is taken at light level; even these are not of ordinary histological sections but chiefly of toluidine blue-stained 1 μ Epon sections. Histochemistry is almost unrepresented. One can only describe the dust cover and title as grossly misleading.

The preparation methods are slightly different from those in general use. Tissues were fixed in 6.25 per cent glutaraldehyde and 2 per cent acrolein in phosphate buffer and postfixed in 2 per cent osmium tetroxide. The first solution seems rather concentrated. It is difficult to say whether this or some other factor is responsible for the very dark and grainy appearance of many of the photographs. The quality of the latter varies. A few are excellent, most are only average and a fair number are distinctly poor. The text is generally helpful but sometimes excessively brief. The wisdom of printing the text in both English and French is doubtful. It must have added to the cost. A more extended text in one language would have been preferable in places.

On the whole this is a disappointing first volume; it does not live up to the claims made for it.

R. BARER

BIOCHEMISTRY OF ANDROGENS

The Androgens of the Testis

By Kristen B. Eik-Nes. Pp. xi + 249. (Dekker: New York, March 1970.) \$14.75; 140s.

THIS monograph discusses the quantitation of testosterone and androstenedione, and attempts to summarize present knowledge of how the vertebrate testicular androgens are formed in the male gonad. It gives the mechanisms responsible for the formation of these androgens and a good chapter on how they are broken down in the body.

The book commences with a chapter on the "Synthesis and secretion of Androstenedione and Testosterone" by Professor K. B. Eik-Nes which gives an excellent picture of the steroid enzymes involved in the synthesis of androstenedione and testosterone.

The section on the formation of oestrogens by the male gonad is interesting. There is no doubt that there may be species differences here, and there seems to be good evidence today that secretion of oestrogens in man by the testes is really insignificant and they may not be secreted at all, being totally produced by peripheral conversion of androstenedione. The next chapter on the "Catabolism of Testosterone and Androstenedione" describes fully the principal catabolic sequence and the end products of catabolism, and discusses the regulation of androgen catabolism and catabolic hormone activity. This is followed by a chapter on gonadotrophic regulation of testicular function. The comment made here, that the intact gonadotrophic responsive cell may not be permitted to express its full steroidogenic capacity without gonadotrophic stimulation, and that it was as if the cell was normally damped down and the trophic hormone released the cell from some inhibitory influence, is certainly very interesting. The book ends with a chapter on the "Estimation of Androstenedione and Testosterone by Physicochemical Methods" by Dr H. J. van der Molen. This chapter is well prepared, but unfortunately is far too bulky in the context of the subject matter of this monograph. Every type of method used to measure the androgens is critically reviewed. Unfortunately, one cannot entirely agree with the author's table of merit for the various methods used for measuring androgens—there seems to be a bias towards competitive protein binding techniques. While these techniques may undoubtedly be our best approach today until we produce reasonable specific antisera to testosterone and androstenedione and then perform solid phase radio-immunoassays, the figure

of 2 for saturation analysis against the figure of 6 for electron capture gas chromatography as precision merit figures, is hardly justified. Again, competitive protein binding techniques for testosterone using sex binding globulin have not been entirely satisfactory in many laboratories, and problems have occurred with high blanks.

In spite of a certain appeal of this book, because it brings together a lot of information about testosterone and androstenedione, it fails to portray the fascinating story of vertebrate testicular androgens. While some of these more interesting aspects are to be found in the book, they are only very briefly touched on. A small section discusses the secretion of androgens throughout life, but only cursory mention is made of the critical events of Mullerian and Wolffian duct fusion, and there is no mention at all about the effect of testicular androgens of the sexual differentiation of the brain. Again, one feels more ought to be written about the hormonal control of spermatogenesis, and the hormonal control of the epididymis, because surely the major purpose of the secretion of testicular androgens is to preserve and control spermatogenesis, the chief function of the testes. The possible physiological significance of di-hydrotestosterone and its possible role as an active form of testosterone in some androgen-sensitive organs is again treated very lightly.

Nevertheless, the chief concern of the book is the biochemistry of androgens, so it should be of interest to graduate students working in endocrinology of the testis and to post-doctoral fellows interested in reproductive endocrinology and steroid biochemistry. Unfortunately, besides its faults it is rather expensive for its 250 pages.

DONALD EXLEY

INSECT TAXONOMIST'S GUIDE

Taxonomist's Glossary of Genitalia in Insects

Edited by S. L. Tuxen. Second enlarged edition. Pp. 359. (Munksgaard: Copenhagen, 1970.) 200 D kr.

THE publication of the first edition of this book in 1956 was something of a landmark in taxonomy because, for the first time, taxonomists had a convenient reference book to the numerous terms applied to the genital structures of insects. The second edition is much enlarged, consisting of 359 pages against the original 284, and all the chapters have either been revised by the original author or revised or re-written by new authors, all of whom are recognized authorities. The original plan of the book remains the same, the principal part consisting of two sections, the first being the description of the genitalia of both sexes of representatives of the various orders, while the second consists of a glossary.

The present work does not aim to attempt the impossible and to try to establish a common nomenclature, although a useful feature in the glossary is that terms applied to homologous structures are indicated. The value of the work lies in enabling a taxonomist to follow descriptions of the genitalia of insects whether he be a specialist or a general taxonomist; it will also be useful to comparative morphologists as a ready reference to some of the range of genital structures.

The 248 text figures, by the authors of the various chapters, are necessarily individualistic, but all are excellent and well labelled. After the description of the male and of the female genitalia of representatives of the particular order, the legends for the figures are given, followed by short references. The abbreviations are explained in one of the initial pages of the book, but additional abbreviations are given for the Hymenoptera.

As with the nomenclature of wing venation, specialists in various orders have tended to apply different terms to homologous structures of the genitalia, while comparative morphologists have attempted to apply terms thought to be indicative of the origin of the structures. Together

with the complexity of most insect genitalia this has resulted in an unnecessary proliferation of names; the most valuable service this work may well achieve is to discourage further coining of terms, and, even better, to reduce the present number. The book is essential to all working taxonomists.

A. BRINDLE

BABOON ANATOMY

Primates

Comparative Anatomy and Taxonomy. Vol. 8: Cynopithecinae. By W. C. Osman Hill. Pp. xix + 680 + 36 plates. (Edinburgh University: Edinburgh, April 1970.) 340s.

I HAVE had the pleasurable task of reviewing the previous volumes in this formidable series and was therefore alarmed to find the number 8 embossed on the latest volume. Whatever happened to volume seven? The first few lines of the preface explains all: volume seven which deals with the macaques and mangabeys is scheduled for 1971; volume eight, bearing the somewhat unfamiliar subtitle "Cynopithecinae" is, for reasons of expediency, out of line. This seems a good decision, bearing in mind the present great interest in the African ground-dwelling Cercopithecinae. Volume eight embraces the genera *Papio*, *Mandrillus* and *Theropithecus* and their extinct ancestors.

We have become so conditioned to the invariability of Dr Osman Hill's scholarship that, with each new volume, it is necessary to remind ourselves of the immense knowledge that has gone into its preparation. Once again, volume eight is an impeccably accurate accumulation of the facts of morphology and taxonomy, but one might wish that behaviour was equally well represented. Possibly this is akin to wishing that Darwin had possessed Mendel's and De Vries' knowledge of the hereditary units. Each generation of scientists contributes to the sum total of knowledge in terms of his own particular expertise. Osman Hill is one of the last of a long line of distinguished primate morphologists and his like, as the saying is, may not be seen again.

Sometimes progress in science is more of a threat than a promise. While scientific progress has its undeniable advantages for mankind, it also has a most depressing effect on the tried and true methods of the basic sciences. If it were true that what is new is necessarily good and what is old-hat is (to use a phrase popular among scientific administrators) unfundable, then the scientists would have no complaint, but is it true? When the electron microscope was introduced, a research project with the light microscope became a non-event, so much so that no ambitious graduate student would willingly opt for the latter if he could possibly climb on the bandwagon of the former. How much information is being missed at cellular level while the eyes of the scientific world are scanning the sub-molecular structure of the cell membrane and its processes? It would be serious indeed if research in primate morphology and the functional interpretation of its discoveries were shunted into the "unfundable" category by the preferentials of new, and as yet unproven, methodologies.

Hill's latest volume will inevitably reach a larger audience than his previous volumes because it deals principally with the baboons which enjoy (if that is the word) a high degree of popularity in the medical research field. For this reason it is perhaps unfortunate that Hill's classification is not wholly in line with recent trends in primate systematics, which is to synonymize the species of *Papio*. It is now recognized that *Papio* is a single polytypic species with morphologically different subspecies interbreeding wherever they meet. Modern taxonomic thought is divided between retaining two species—*P. hamadryas* and *P. cynocephalus*—or treating

them as conspecific; in which case the specific name would have to be *P. hamadryas* under the Priority Rule. Common sense, for the time being, is in the ascendancy and confusion among the ranks of non-taxonomists is postponed by admitting two species—the sacred baboon (*P. hamadryas*) and the common baboon (*P. cynocephalus*). Recent classifications also favour the inclusion of the drills and mandrills in the genus *Papio*, on grounds that are almost wholly pragmatic, and, in my opinion, biologically invalid. Fortunately, the informational content of volume eight of *Primates* is in no way affected by these nomenclatorial niceties.

The book is profusely illustrated with line drawings and black and white plates. It would be nice to see a few more colour plates; the Devil (or in this case the popular monthly glossies) always seems to have the best tunes. What a pity it is that colour is not as readily available to scientists, who often require it to prove their point, as it is to purveyors of sex magazines whose point is already proved.

Anybody who possesses the first six volumes of this series will need no urging to add volume eight to their collection. But for those who have not yet invested in *Primates* and have a special interest in the African Cercopithecinae, this new volume will provide a permanent, accurate, up to date source of historical, morphological and physiological information collected by the acknowledged expert in the field of primate morphology.

JOHN NAPIER

GUIDE TO BRITISH LICHENS

Introduction to British Lichens

By U. K. Duncan, assisted by P. W. James. Pp. lxxiv + 292 + 128. (Buncle: Arbroath, 1970.) 70s.

THE principal object of this book is to assist students and beginners to identify lichens. It does not attempt to be a definitive lichen flora of Britain, and, indeed, it only includes 65 per cent of the species recorded for this country (the other 35 per cent being lichens which the beginner is very unlikely to encounter).

There is no doubt that the book will succeed admirably in its object. It will be extremely useful in lichen identification because the keys are particularly good and are easier to follow than in any other relevant lichen flora; there are adequate descriptions of each species. Unfamiliar terms are explained both in a glossary and in a detailed account of lichen structure in which technical words are put into bold type. There are also lists of species found in different habitats—which, again, will be of great assistance.

The chief defect is the illustrations, consisting of 128 pages of line drawings (unfortunately called plates) which are often tiny, many occupying less than half the space available to them on the page. If all but a few of the illustrations had not been published, the usefulness of the book would not have suffered, and its price would have been brought more comfortably down into the range acceptable to students. Another irritating feature is that in the excellent key to genera, there is no indication of the page on which the description of a genus occurs. Unless the reader has an accurate and very detailed grasp of lichen classification, he will have to continually turn to the index to locate the relevant page.

Perhaps the basic problem is that the book is published by a firm which seems not to have much experience in publishing this kind of scientific book. This is a great pity because the book is essentially very useful. Miss Duncan has a remarkably good and critical knowledge of lichens, and, as those who attend British Lichen Society excursions will testify, she is outstandingly good at helping beginners. All students of British lichens should have this book.

D. C. SMITH

FOR GRASS-SPOTTING SWAGMEN

The Grasses of Central Australia

By M. Lazarides. Pp. xi+282. (Australian National University: Canberra, March 1970.) \$10.0.

MR LAZARIDES provides a valuable catalogue of the grass resources of the arid area of central Australia. Designed for use primarily by non-specialist taxonomists, his book comprises a concise, lucid account of the land and vegetation types in the area, followed by an extensive treatment of the grasses.

Grasses make up most of the forage, and must be adequately known before improved management policies can be effected. In an area so inimical to plant growth, efficient exploitation, or conservation, of the grass flora is urgent, and rests on better knowledge of the taxonomy and ecology of constituent species. Lazarides offers a sound guide to both, and his work must be a spur to further research. Though conditions here are so inhospitable, it is galling for a British grass-spotter to note that the grass flora is richer than that of Britain. Many species show fascinating feromorphic adaptations.

The taxonomic treatment of the grasses is preceded by a short, illustrated account of the morphology of the grass plant. The attempt made to simplify this complex subject, without any bending of the facts, is well done. There is a clear generic key.

Descriptions of species occupy most of the book. Each consists of a general account of habit, with notes on occurrence, distribution and forage value, followed by a detailed "botanic description" of the reproductive morphology. There are good photographs of almost all species, often showing spikelet details. Accounts are rendered less awesome, perhaps, to the non-taxonomist, by relegation of nomenclatural details and citations to an appendix.

Minor criticisms which can be levelled at this book arise from the author's praiseworthy attempt to write for a wide audience. There is much to be said for his alphabetical arrangement of genera and species, but could not one page be spared for listing genera into tribes? The first item in the species descriptions is the common name, followed by the Latin binomial. This may make for readier reference by pastoralists, but the author confesses that to achieve this he invented many common names. Regrettably, these are not identified for the sake of later folk studies. They are merely English names which may become common only through the relentless action of natural selection. Brawny cattlemen, perceiving *Eragrostis* from their galloping steeds, may scorn to cry "Cuming's love-grass"!

This notable contribution to the literature of the Australian arid regions is a desideratum for all botanical swagmen.

P. M. SMITH

PLANT CHEMISTRY

Phytochemical Phylogeny

Edited by J. B. Harborne. (Proceedings of the Phytochemical Society Symposium, Bristol, April 1969.) Pp. xiii+335. (Academic: London and New York, June 1970.) 110s; \$5.50.

THIS volume is the latest in the series devoted to the annual symposia of the Phytochemical Society. The book has a wide sweep with articles ranging from "The Origin of Plants" by P. Echlin, to "Secondary Constituents of Aquatic Angiosperms" by J. W. McClure, taking in *en route* such widely divergent topics as the controversial relationship between bacteria, blue-green algae and chloroplasts (N. G. Carr and I. W. Craig) and the straight organic chemistry of modern and fossil plant resins (B. R. Thomas). Two important contributions come from the Cell Research Institute and Department of

Botany, The University of Texas, Austin, the birthplace of modern phytochemistry in the United States; these contributions "Molecular Approaches to Population Problems at the Intraspecific Level" (B. L. Turner), and "Intraspecific Variation of Sesquiterpene Lactones in *Ambrosia* . . ." (T. J. Mabry) demonstrate how a beaver-like accumulation of data can be used imaginatively to open up new aspects of phytochemical phylogeny. R. L. Watts contributes a thoughtful and readable study of the basic principles, both genetic and biochemical, which underly the importance of studying proteins in relation to plant phylogeny. It is an appropriate time to spell out the reasons for continued studies on amino-acid sequences, now that sequencing is becoming almost a routine performance in modern laboratories devoted to protein chemistry. Watts's contribution is followed by a description by D. Boulter *et al.* of their sequence studies on cytochromes *c* from higher plants.

Both Echlin and Chaloner and Allen (palaeobotany and phytochemical phylogeny) point out the dangers which can befall the unwary in dealing with palaeobiology; for example, the ease with which it is possible to confuse artefacts with microfossils and the danger in ascribing the origin of a biochemical extracted from a coaly residue to the source plant: it could be a product of microbial degradation which had occurred before fossilization. Other chapters are concerned with sporopollenin (Shaw), the peculiar and extremely resistant polymer of the pollen wall which is thought to arise by polymerization of pollen carotenoids; with lipids of various photosynthetic organisms (B. W. Nichols), and with environment and enzyme evolution in plants (H. W. Woolhouse), a subject which needs considerably more study before its full implications are apparent. Finally, but by no means least, is a mature article by S. Bartnicki-Garcia on the importance of cell wall composition in fungal phylogeny; in relation to this his final conclusion that "Barring an unforeseen breakthrough in palaeomycology, the use of morphology in conjunction with biochemical data represents the only hope for reconstructing key events in the evolutionary history of the Fungi", is particularly significant.

I congratulate the participants in producing such an effective series of reports: the book represents phytochemistry at its best and any non-specialist reading this book is bound to be impressed by the distance modern phytochemistry has travelled during the past decade. The phytochemist of the 1970s is a person of wide interests who is successfully responding to the challenge of the new ideas developing in biology and chemistry, and who is meeting fresh problems with the intelligent use of modern equipment ranging from the mass spectrometer to the computer.

The indefatigable secretary of the Phytochemical Society, J. B. Harborne, has edited this volume with his usual professional thoroughness and has included four indices: author, chemical compound, genus and species, subject. The book seems singularly free from factual errors, although it seems inevitable that a book I review will have a carotenoid structure wrong (p. 5). The production of the book is up to the publisher's usual high standard.

T. W. GOODWIN

ELECTROCHEMISTRY

Modern Electrochemistry

An Introduction to an Interdisciplinary Area. By John O'M. Bockris and Amulya K. N. Reddy. Vol. 1: Pp. xxxii+1-622. 165s. Vol. 2: Pp. xxvii+623-1432. 190s. (Macdonald: London, July 1970.)

THESE two substantial volumes provide a novel and stimulating approach to the teaching of electrochemistry

as an interdisciplinary subject in a modern context. For many years, there has been a clear need for a teaching textbook with a non-specialist approach and with an emphasis on modern concepts, and this work is aimed at this gap. The authors maintain that the development of electrochemistry has been seriously retarded by an over-emphasis on the classical thermodynamic approach, and they seek, with crusading zeal, to correct this. As a result, a number of old battles are fought and the sound of this strife may well puzzle younger ears. The importance and utility of the Nernstian heritage are purposely neglected, and at times derided, and, in this sense, the book is unbalanced. The positive features of the work, however, are many and must be stressed.

The first volume gives a lucid and lively description of ionic solutions and it includes a large section on molten salts and glasses. The reader is led gently and expertly through a long series of interlocking discussions on the structure of water, ion-solvent interactions and ion-ion interactions in dilute and in concentrated solutions. The approach emphasizes modern, mechanistic descriptions, it is constructively critical and deals very effectively with the quantitative aspects. The material is built up gradually from an elementary level with a certain amount of repetition, to meet the needs of beginners and of those from outside the field who are seeking electrochemical expertise relevant to their own discipline; thus the book is long, but it is not tedious. Each chapter is divided into short, headed and indexed sections, and good lists of further reading material are provided at appropriate intervals.

The second volume deals in a similar manner with electrified interfaces and electrode processes. The content is generally excellent; for example, it includes a treatment of the semiconductor-electrolyte interface and of electrokinetic properties, and a discussion of electrocatalysis, electrodeposition, corrosion, fuel cells and batteries, but it is surprising in a modern text that no space is found for organic electrochemistry, an important and rapidly growing subject in which many practitioners are non-electrochemists in need of guidance. Much of the material lies close to the research interests of the authors, and, inevitably, their particular viewpoint on a number of topics is apparent. Their preference occasionally becomes obtrusive in the selection of scientists to be described as the most important contributors to the development of a particular branch of the subject.

The racy, colloquial style is easy to read and is seldom clumsy or obscure. The authors' predilection for coining new names is usually harmless, but there is no justification for neglecting the established terms cathode, anode, reduction and oxidation and substituting electron source electrode, electron sink electrode, electronation reaction and deelectronation reaction. The scope for electrochemistry and its importance in our technologically developing society is discussed at points throughout the books in a stimulating manner, although some of the examples used are very speculative. I am sure this work will substantially aid the vigour and lucidity of the teaching of electrochemistry at the undergraduate and postgraduate levels, and it will be welcomed by many research workers from other fields. A. BEWICK

BASIC QUANTUM MECHANICS

Quantum Mechanics

An Introduction. By J. G. Taylor. (Unwin Studies in Physics.) Pp. 207. (Allen and Unwin: London, June 1970.) 50s boards; 25s paper.

THIS book is meant to cover a one year course for students who are meeting quantum mechanics for the first time, and who will not necessarily specialize in theoretical

physics. This sensible aim is roughly that of the existing books by Matthews, Strauss and Ziock, for example. Taylor's book stands the comparison well. To begin with, its price is right: you get a lot of quantum mechanics for the price of the paperback edition. For the most part, it is a businesslike presentation of basic quantum mechanics. The book begins, very wisely these days, with a concise summary of classical mechanics. The Schrödinger equation and interpretative postulates are then introduced, and the standard one- and three-dimensional potential problems are mentioned. Time-independent and (an omission in Matthews) time-dependent perturbation theory is discussed, as in Strauss, in the context of atoms and molecules. The sixth chapter contains a much fuller account of scattering theory than either Matthews or Ziock offer, using the Green function method, and the book concludes with a pointer towards the Dirac equation and field theory.

To me the book seems to be particularly suitable for the student who is, in fact, going to specialize in theoretical physics; for such, the two or three page hints about variational principles, linear self-adjoint operators on the Hilbert space $L_2(\mathbb{R}^3)$, the rotation group and the analytically continued S-matrix would make sense. Although there are some useful analytical problems at the end of each chapter, there are very few numerical ones (as compared with Ziock or Strauss); also one might have reservations on whether the very brief discussion of non-relativistic spin formalism is adequate, especially when compared with Ziock's treatment. But Taylor's book, good value for money, is well worth considering.

I. J. R. AITCHISON

ABSORBED ENERGY

Photophysics of Aromatic Molecules

By John B. Birks. (Wiley Monographs in Chemical Physics.) Pp. xiii+704. (Wiley (Interscience): London and New York, May 1970.) 210s.

DR BIRKS is a well known experimental physicist in the field of phosphorescence and fluorescence in liquids and solids and he is especially interested in the relative efficiencies and rates at which these processes occur. Photophysics as opposed to photochemistry is not a word I have met previously. The author explains it as being concerned with the physical effects of non-ionizing radiation, but he has restricted himself largely to the subsequent history of the absorbed energy. He does not cover in any detail allied physical properties such as zero-field splitting and the electron resonance of triplet states, the absorption spectra of excited molecules, flash photolysis, and the like. Not that these subjects are totally ignored, but they are only mentioned where they relate to the principal themes of fluorescence, radiationless decay, internal conversion and phosphorescence. Photoconductivity, for instance, is covered in less than two pages.

"Aromatic Molecules" in the title also has a narrow meaning, because heterocyclic molecules, and therewith all σ lone pair excitations, are largely excluded. But in the selected field of benzene and the condensed aromatic hydrocarbons such as anthracene and their substituted derivatives the coverage is thorough. Much information is given in tabular form with consistent units and nomenclature, while the text comments helpfully on superseded work, and cases where impurities are suspected or imperfect techniques were used. Anybody requiring up to date numerical values of fluorescence or phosphorescence yields or life times, quenching constants, spectral peak energies or similar information for any carbocyclic aromatic molecule, its excimers, its donor-acceptor complexes in either crystal, glass or solution states is likely to find a reliable number here.

All this is excellent for those with closely related

interests to the author. I do not belong to this class, but am an interested worker in a neighbouring field who hoped to look over the hedge with interest and profit. In this I was a little disappointed. Birks himself says "Theory has been kept to the essential minimum", and while he is right not to repeat well known quantum theory, he does not use it much either. Paradoxically, the account would be easier really to comprehend if the concepts were less simply expressed. The simple perimeter free electron orbital model language chiefly used is less informative than state descriptions based on molecular point groups, especially when the former is introduced by figure 1-4, where the idea of nodal planes seems to be applied to many electron states rather than to one electron orbital. And while spin-orbit coupling is all important in radiative and radiationless singlet-triplet transitions, the discussion is largely qualitative, the energy term appearing twice as H_{so} and never as ζ l.s. or more complicated expressions.

This, then, is an important new monograph for which all active in the field will thank the author for his clear display of the known facts. It is an essential purchase for all relevant libraries. In considering its suitability for students, young or old, I am driven in honesty to report that I felt my knowledge had been increased more than my real understanding.

D. H. WHIFFEN

INTERNATIONAL MATHS

Mathematics Applied to Physics

Edited by E. Roubine. Pp. xvii + 610. (Springer-Verlag: Berlin and New York, 1970.) 58 DM; \$16.

A UNESCO sponsored congress on science teaching, held at Dakar in 1964, "agreed to recommend, as a priority measure, the preparation of a university syllabus of mathematics to meet the needs of physicists". This book is apparently the outcome of that decision, although it is clearly not a syllabus, being a collection of connected articles on various branches of mathematics, nor is it a textbook designed to meet the needs of such a syllabus, because several chapters are rather too concise to be easily followed by students quite unfamiliar with the material.

Each chapter is an admirably clear, up to date, and, in nearly every case, a readable account of its subject; chapter one confines itself to listing definitions and theorems; chapters four and ten are more restricted in scope than the rest and so are able to provide more leisurely treatments, even giving a few proofs; the remaining chapters find a compromise between these extremes. The chapters are largely independent of each other: the few cross-references give the impression of being afterthoughts.

The result of a collaboration between authors from seven countries, the book seems, perhaps inevitably, slightly uncertain of its level. To take a trivial example, it is not assumed that the reader knows what a differential equation is, although the term Hilbert space is used several times without definition.

The needs of physicists are seen in fairly classical terms: more than half of the book is devoted to differential equations. The final chapter, called "Quantum Mechanics", is considerably shorter than any of the others, and is, in fact, an elementary account of group representations and angular momentum in quantum mechanics.

If allowance is made for its classical emphasis, this book succeeds very well in providing a "syllabus plus some of the important details" covering the basic mathematical requirements of many branches of physics. It treats in an economical manner several important subjects, providing references to more detailed treatments where necessary, and so should also provide a useful work of reference.

J. UNDERHILL

Short Notices

Science, Industry and Society: Studies in the Sociology of Science. By S. Cotgrove and S. Box. Pp. xii + 211. (Allen and Unwin: London, July 1970.) 50s boards; 30s paper.

A HACKNEYED phrase in the sociology of science is that nearly every scientist who has ever lived is alive today. Stephen Cotgrove and Steven Box inform us of that in the opening sentences of the cover notes, the preface and chapter one of their book. That is a pity, because the book contains a much more original insight into some of the problems currently being faced by scientists—the influences of industry on the universities, the satisfaction and dissatisfactions of working in a laboratory and the gulf between academic and industrial scientists. The basis of the book is a series of questionnaires sent to scientists in universities and in industry, and the facts are presented in a comprehensive if rather dull manner. *Science, Industry and Society* stands, however, alongside Stephen Cotgrove's *Science of Society* as an important contribution to the sociology of science.

SI Units and Conversion Tables: A Practical Guide for Scientists. By M. N. Hughes, A. M. James, and N. R. Silvester. Pp. 23. (Machinery: Brighton, 1970.) 4s 6d.

THIS booklet is not designed to add more polemic to the discussion of the SI system but simply presents, in its uncompromising finality, a compilation of the symbols and units which constitute this system and the conversion factors relating it to its imperfect predecessors. The work seems to be aimed primarily at physicists and chemists, and yet anybody with editorial experience of the biological literature will know that the largest remaining concentration of the unconverted is to be found among the life scientists. This booklet should give them cause for introspection: not for long will they hold out against the schools on the one hand and technology on the other where the system is firmly implanted. One can comprehend but not condone the biologist's affection for units such as Å, the Svedberg and mmHg. With all respect, Ångström and his colleagues Gauss, Maxwell, Oersted and others have had a good innings; surely even the biologists can be prevailed upon to acclaim the new presidium: Newton, Weber, Joule and their friends.

Red Data Book. Vol. 5: Angiospermae. By R. Melville. (International Union for Conservation of Nature and Natural Resources Survival Service Commission: Morges, Switzerland, 1970.) SFr. 30; \$7.00; 60s.

THE status of many threatened plants is so uncertain that the International Union for Conservation of Nature and Natural Resources has deemed it wise to publish before completion the latest volume of its catalogue of rare and vanishing species. As more information becomes available further sheets will be prepared for this loose-leaf volume to add to the sixty-eight endangered angiosperms that Dr Melville has traced so far. Non-specialists need have no fear that this information will be beyond their comprehension; plants are described in the straightforward language of school botany. There are also, whenever possible, notes on distribution, habitat, biological value and so on. Remedial measures recommended include the preservation of Mount Athos in Greece as a national park free of grazing animals, so that *Centaurea athoa*, yellow Athos knapweed, may thrive again.

Correspondence

Semiconductor History

SIR,—I recently read the article, "Relationship between Science and Technology", by M. Gibbons and C. Johnson, published in the July 11 issue of *Nature* (227, 125; 1970). I found it very interesting. However, it contains several errors of fact.

In the section "Development of New Techniques" the statement is made that the need for radar detectors prompted much government sponsored work in the United States, principally at Purdue and Cornell Universities. In fact, the renaissance of the crystal detector was due to work on silicon carried out in Bell Telephone Laboratories prior to the war by R. S. Ohl, G. T. Southworth, A. P. King and others under the leadership of H. T. Friis. This work preceded the government programme by several years. The government programme in this country was centred at the Radiation Laboratory at the Massachusetts Institute of Technology as described by Torrey and Whitmer¹. Supporting programmes on germanium were carried out later at Purdue University under the leadership of the late Dr Karl Lark-Horovitz and on silicon at the University of Pennsylvania under Dr Frederick Seitz. I am not aware of a similar programme carried out at Cornell University. Finally, R. S. Ohl, H. C. Theuerer and myself were never employed at Purdue University. Our work in this field was all done at Bell Telephone Laboratories^{2,3}.

Yours faithfully,

J. H. SCAFF

Bell Telephone Laboratories Inc,
Murray Hill,
New Jersey 07974,
USA.

¹ *Crystal Rectifiers* (Radiation Laboratory Series, McGraw Hill Book Co., New York, 1948).

² Ohl, R. S., and Scaff, J. H., *Bell System Tech. J.* 26, 1 (1947).

³ Scaff, J. H., *Metallurgical Trans.*, 1, 561 (1970).

Pop Charts for Science

SIR,—Mr E. Garfield's paper (*Nature*, 227, 669; 1970), though rational, is disturbing. While the quotation-coefficient of a paper, if one may call it that, is to some extent a measure of the use made of its content, it would be unfortunate if the support a scientific author receives were to be made conditional on his current position in the charts. One can imagine reasons why papers in industrial chemistry might rate higher than those in, say, topology or palaeontology. In fact, were we to confine our analysis to papers published in China, the chart leader would no doubt be Chairman Mao.

In fact, the analogy from pop music raises the covers from some of the abuses we might expect (and even, while waiting for our Nobel Golden Disk, indulge in): commercial concerns dedicated to lobbying, so that our rating might be raised? Promotional quotation-data on the relative one-upmanship of particular journals? In California nothing is impossible.

We must all accord respect to Mr Garfield's ingenuity in devising a means whereby an unskilled staff can score

publications for reference: that our capacity for publicity should become a measure of our worthiness of support is, however, a trifle *simpliste*. I am reminded that, according to anecdote, one of the centurions supervising the Crucifixion remarked to his comrade, "I hear he was a great teacher". "Yeah", replied the other, "but he never published anything." The fact that his graduate students did would elude Mr Garfield's net.

Yours faithfully,

ALEX COMFORT

University College London,
Gower Street,
London WC1E 6BT.

Disputed Pronoun

SIR,—Hammerton¹ has attempted to refute Chomsky's² assertion that, in the sentence "Learning that John had won the race surprised him", the pronoun *him* cannot refer to John. The refutation, claimed to be significant at the level of one part per million, was based on the responses of twenty people who read an *ad hoc* paragraph containing the sentence in question. The respondents all felt sure that, in the given context, *him* referred to John. It is my contention that it was an agreement of desperation. If *him* did not refer to John the paragraph would be meaningless. Hammerton's subjects had the choice of ignoring Chomsky's rule or admitting that they did not understand what was otherwise a trivial paragraph.

I also have done a poll. Hammerton's paragraph was typed and offered to twelve subjects to read. They were then asked what they thought of the penultimate sentence (the one in question). A typical reply was, "I thought I was understanding the passage till I got to that sentence. Then I had to start the paragraph again to verify who John and Bill were". All twelve readers agreed that substitution of *he* for *John* would improve the sentence and make the paragraph clear on first reading.

However, when a name denotes both a person and his public image Chomsky's rule may be relaxed. For example, in the sentence, "Learning that Heath had won the election surprised him", *him* can refer to Mr Heath, but not *Heath*. English permits Mr Heath to say "Heath won" but it does not permit John to say "John won" unless John is a king or a second person with that name.

After we understand the linguistics of well constructed sentences will be early enough to tackle sentences like this one or Hammerton's paragraph.

Yours faithfully,

EDWARD ARGYLE

Dominion Radio Astrophysical Observatory,
Box 248,
Penticton, BC, Canada.

¹ Hammerton, M., *Nature*, 227, 202 (1970).

² Chomsky, N., *Language and Mind* (Harcourt, Brace and World, New York, 1968).

Obituaries

Dr H. Boyko

HUGO BOYKO, widely known as an ecologist of arid regions and as the founder and president of the World Academy of Art and Science, died in Israel on May 26, 1970. He was 78.

Boyko's early academic associations were with the University of Vienna, but before the Second World War he emigrated to Palestine. His early publications gave a comprehensive picture of what was then called "plant sociology". After emigration his preoccupation with the ecology of arid zones in the Mediterranean area first led him to investigate the role of plants as quantitative climate indicators and the geo-ecological law of distribution. He was particularly interested in the relations of plant ecology with hydrology and hydro-engineering, and indeed his later work concentrated chiefly on the use of salt water for the reclamation of arid zones. In 1950 he was appointed by UNESCO as a member of the international advisory mission for arid zone research, and he continued to investigate the possibilities of sea-water irrigation for the rest of his life. One practical consequence was the transformation of the desert area of Elath into a garden and resort. Boyko's ideas were set out in two books, *Salinity and Aridity* (1966) and *Saline Irrigation for Agriculture and Forestry* (1968).

In the course of his travels for this purpose, he was able to assemble a large herbarium, which covered specifically the plants of the Middle East.

Boyko's humanitarian leanings were also made explicit in his desire for international cultural cooperation. In 1956 he gave a lecture on the need for an international academy of arts and sciences at the International Conference on Science and Human Welfare in Washington. By 1960 his efforts had culminated in the founding of the World Academy of Art and Science, which has now grown to include more than three hundred fellows chosen on the strength of their intellectual achievements and their concern for human welfare. As president of the academy, Boyko was taking part in this year's conference on the environment and on society in transition, in New York, just a few weeks before he died.

Professor S. J. Folley

SYDNEY JOHN FOLLEY, FRS, died in hospital on June 29. He was 64 years old. He had been head of the physiology department of the National Institute for Research in Dairying (University of Reading) since 1945 and a research professor of the university since 1964.

He was born in Swindon, Wiltshire, and received his early education in that county. With a county university

scholarship he entered the University of Manchester in 1924, graduating in 1927 with first class honours in chemistry and with the award of the Mercer Scholarship.

After a year's postgraduate research in colloid chemistry he transferred to the physiology department and became personal assistant to Professor H. S. Raper. His first official university post was that of assistant lecturer in the biochemistry department at Liverpool. In 1932 he went to the NIRD as research assistant to the recently appointed physiologist, who resigned in 1933, leaving Folley and a young technician as the entire staff of the embryo physiology department.

Folley's interest in the endocrinology of mammary function in relation to dairying was soon aroused, and with improving laboratory and animal facilities and increasing scientific assistance his original and active mind began to probe deeply into this field. He was given charge of a section of lactational physiology in 1939. This evolved, after the war, into a full physiology department with a staff of twelve and most of the facilities necessary for experimental work on animals of all sizes.

There followed in his department an exciting period of research in which the effects of thyroxine and of pituitary hormones on milk quantity and quality, and also those of stilboestrol on mammary growth and milk production in virgin heifers, were quantitatively studied and the new knowledge firmly established. It was also demonstrated ("the first major experiment by the use of ^{14}C in Britain") that the short-chain fatty acids of milk fat were, in the ruminant, built up in the alveolar cells of the udder largely from the acetate taken up from the rumen into the circulating blood. Valuable findings were also made on the minute structure of the milk-secreting tissues and on the neuro-endocrine control of milk ejection based on a delicate method devised in his laboratory for the assay of the milk-ejection hormone, oxytocin.

These and many other physiological and biochemical problems were successfully attacked by Folley, assisted by his colleagues and visiting scientists, bringing distinction to his department, his institute and himself. He was elected a Fellow of the Royal Society in 1951 and his work has received further wide recognition both at home and abroad. In 1964 he was made an honorary doctor of veterinary medicine at the University of Ghent and he received the Dale Medal in 1969.

In his later years his failing eyesight, which deteriorated rather rapidly so that he was unable to read or work with his own hands in the laboratory, made him increasingly dependent on colleagues. But by means of tape records and other aids he was able to keep well abreast of advances in his field of research, and the successful pursuit of his work to the end was an exemplary manifestation of his courage and the devoted support of his wife.

Announcements

University News

Professor Robert E. Davies, professor of biochemistry at the University of Pennsylvania School of Veterinary Medicine, has been appointed Benjamin Franklin professor of molecular biology.

Dame Mary Cartwright, reader in the theory of functions in the University of Cambridge, will visit the Institute of Mathematics of the Polish Academy of Sciences and other Polish universities as Royal Society Leverhulme visiting professor.

Dr J. C. Amson, University of St Andrews, has been awarded a research grant by the Centre for Environmental Studies, London, to study the application of advanced mathematical theories to the structure, growth and stability of urban systems.

Dr D. Lack, director of the Edward Gray Institute of Field Ornithology, University of Oxford, has been appointed Royal Society visiting professor to the University of the West Indies, Jamaica, for the academic year 1970-71.

Appointments

Professor John Heslop-Harrison will succeed **Sir George Taylor** as director of the **Royal Botanic Gardens, Kew**, when Sir George retires in May 1971.

Mr Robert Wood has been appointed director of studies at the Overseas Development Institute in succession to Dr Tom Soper.

Mr W. E. Jones, chief agricultural adviser to the Ministry of Agriculture, Fisheries and Food, has been appointed first director general of the MAFF Agricultural Development and Advisory Service which is to start on March 1, 1971. **Major E. S. Dobb**, at present director of the Agricultural Land Service, and **Mr W. R. Smith**, at present director of the National Agricultural Advisory Service, will be deputy directors general of the new service.

Miscellaneous

The **J. J. Thompson award** of the **Institution of Electrical Engineers** has been won jointly by **Dr H. G. Lubszynski**, **Dr B. J. Mayo**, **Mr J. Wardley** and **Mr N. C. Barford**, all of EMI, for their paper "New All-electrostatic Vidicon".

Professor B. E. C. Nordin, head of the MRC Mineral Metabolism Unit, Leeds General Infirmary, has won the first **International Health Foundation award**, for his paper "The Clinical Significance and Pathogenesis of Osteoporosis."

The **Hanbury Memorial Medal** of the Pharmaceutical Society of Great Britain, awarded biennially in memory of the nineteenth century pharmacist Daniel Hanbury, has been won by **Professor R. B. Woodward**, Donner professor of science at Harvard University. Professor Woodward will deliver the memorial lecture on "Recent Advances in the Chemistry of Unnatural Products", at the School of Pharmacy, University of London, on November 4.

The Council of the Royal Society has awarded four **Royal Society Leverhulme scholarships**: to **Mr Patrick Duncan**, University of Oxford, to study the habitat utilization of the topi in the Serengeti National Park; to **Mr B. J. T. Jones**, University of Cambridge, to investigate at Mount Stromlo Observatory phenomena in the southern skies relating to the formation and properties of galaxies; to **Mrs P. J. Edwards**, University of London, to study the freshwater fishes of New Guinea; and to **Mr D. J. Maberley**, University of Oxford, to carry out an ecological investigation in the mountains of East Africa and Madagascar of thick stemmed vegetation with special regard to its origin.

ERRATUM. In *Nature* of August 29, the article entitled "Membranes Dismembered" (227, 888; 1970) was attributed to our Microbiology Correspondent. It was, of course, by our Molecular Biology Correspondent.

ERRATUM. In the review, "Weddell's Voyage South", by H. G. R. King (*Nature*, 227, 973; 1970), the words "chronometer sand tables" in lines 16 and 17 of the second paragraph should read "chronometers and tables", and the word "Indies" in line 24 in the same paragraph should read "Indians".

International Meetings

September 7-12, **Hormonal Steroids**, Hamburg (Dr J. Tamm, 2 Medizin, Universitätsklinik, Martinistrasse 52, 2000 Hamburg 20, Germany).

September 9-11, **British Association for Crystal Growth Conference**, Bristol (Dr D. Elwell, Physics Department, Portsmouth Polytechnic, Park Road, Portsmouth, Hampshire).

September 9-11, **British Pharmacological Society Meeting**, London (Dr P. J. Piper, Department of Pharmacology, Royal College of Surgeons, Lincoln's Inn Fields, London WC2 3PN).

September 14-16, **Institute of Electrical and Electronics Engineers Group on Antennas and Propagation Meeting**, Columbus, Ohio (William D. Stuart, Battelle Memorial Institute, Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201, USA).

September 15-17, **US National Committee of URSI Meeting**, Columbus, Ohio (William D. Stuart, Battelle Memorial Institute, Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201, USA).

September 17-19, **Prostaglandins**, New York (The Executive Director, New York Academy of Sciences, 2 East Sixty-third Street, New York, NY 10021, USA).

September 22, **Technology and the Environment**, London (Information Department, Confederation of British Industry, 21 Tothill Street, London SW1).

September 22, **Analysis and Control of Pollution**, Bristol (Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF).

September 24, **Air Coolers**, London (Conference Publicity Section, Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1).

September 24, **The Feeding Habits of Schoolchildren**, London (D. J. Vogler, The National Dairy Centre, 5-7 John Princes Street, London W1M 0AP).

September 29-October 2, **Trunk Telecommunications by Guided Waves**, London (Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

September 30, **Spectra and Structures of Molecular Ions** (Faraday Lecture), London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

October 5-9, **Electron Microscopy Society of America Meeting**, Houston (Carl Tessmer, Section of Experimental Pathology, University of Texas MD Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas 77025, USA).

October 5-9, **Plutonium and Other Actinides**, Santa Fe (F. W. Schonfeld, Los Alamos Scientific Laboratory, PO Box 1663, Los Alamos, New Mexico 87544, USA).

October 13, **Profit from Tribology**, Glasgow (Ministry of Technology, Millbank Tower, Millbank, London SW1).

October 13-16, **Population 70—Family Planning and Social Change**, Tokyo (The Information Officer, International Planned Parenthood Federation, 18-20 Lower Regent Street, London SW1).

October 20-23, **Clean Air Exhibition**, Southport (National Society for Clean Air, 134-137 North Street, Brighton, Sussex).

October 21-22, **British Coal Utilization Research Association Open Days**, Leatherhead (Public Relations Officer, BCURA, Randalls Road, Leatherhead, Surrey).

October 22-23, **Organic Microchemistry and its Applications**, Salisbury (Society for Analytical Chemistry, 9-10 Savile Row, London W1X 1AF).

Sabbatical Itinerants

Entries of this kind can now be found among the classified advertisements

British Diary

Monday, September 7

Basic Concepts in Modern Control Theory (five-day vacation school) Institution of Electrical Engineers, at the University of Birmingham.

Control Mechanisms of Growth and Differentiation (five-day symposium) Society for Experimental Biology, in conjunction with the Society for Developmental Biology, at Eliot College, University of Kent, Canterbury, and Wye College (University of London), Wye, Ashford, Kent.

Computational Physics (three-day conference) Institute of Physics and the Physical Society, at Imperial College, London SW7.

Third International Broadcasting Convention (five days) Institution of Electrical Engineers, and the Institution of Electronic and Radio Engineers, at Grosvenor House, London W1.

Tuesday, September 8

Optical Techniques for Investigations in the Upper Atmosphere (three-day conference) Institute of Physics and the Physical Society, at the University of Exeter.

Wednesday, September 9

Second Particle Size Analysis Conference (three days) Society for Analytical Chemistry, at the University of Bradford.

The Degradation of High Polymers (informal discussion) Faraday Society, at the University of Surrey, Guildford, Surrey.

Sunday, September 13

Second International Conference on Raman Spectroscopy (five days) at New College, Oxford.

Monday, September 14

Aerodynamic Noise (four-day symposium) Loughborough University of Technology, in collaboration with the British Acoustical Society, and the Royal Aeronautical Society, in the Edward Herbert Building, University of Technology, Loughborough.

Photoionization Phenomena and Photoelectron Spectroscopy (three-day conference) Institute of Physics and the Physical Society, Atomic and Molecular Physics Sub-Committee, in collaboration with the Theoretical Chemistry Group of the Chemical Society, at the University of Oxford.

The Economics of Ammonia Production (two-day international symposium) Fertiliser Society, at Shell Centre, London SE1.

The Physics of Non-Crystalline Solids (third international conference) at the University of Sheffield.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Zoological Record, 1967, Vol. 104, Section 19: Mammalia. Compiled by the Staff of the Zoological Society of London. Pp. vi+450. 60s. 1968, Vol. 105, Section 12: Arachnida, together with Merostomata, Pantopoda, Pentastomida, Tardigrada, Myriapoda and Onychophora. Compiled by Ernest Browning. Pp. vi+88. 40s. (London: The Zoological Society of London, 1970.) [108]

The Weir Group—Descriptive Brochure. Pp. 50. (Cathcart, Glasgow: The Weir Group, Ltd., 1970.) [108]

Memoirs of the Royal Astronomical Society, Vol. 73, Part 3: A Catalogue of Early-Type Stars Whose Spectra Have Shown Emission Lines. By Lloyd R. Wackerling. Pp. 158-319. (Oxford and Edinburgh: Blackwell Scientific Publications, 1970. Published for the Royal Astronomical Society.) [108]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 259, No. 828 (6 August, 1970): A Discussion on Determination of Sex. Organized by G. W. Harris and R. D. Edwards. Pp. 1-206+plates 1-23. (London: The Royal Society, 1970.) 140s; \$18.20. [108]

Department of Employment and Productivity. Safety in the Use of Woodworking Machines. (Health and Safety at Work, No. 41.) Pp. 140. (London: HMSO, 1970.) 13s (65p) net. [118]

Putting Paediatrics in Its Place. By Professor Charlotte M. Anderson. (Inaugural Lecture delivered in the University of Birmingham on 5th February 1970.) Pp. 14. What Philosophy is About. By Professor C. H. Whiteley. (Inaugural Lecture delivered in the University of Birmingham on 23 October 1969.) Pp. 14. A View from a Bridge. By Professor Thomas

Patterson Whitehead. (Inaugural Lecture delivered in the University of Birmingham on 18th November 1969.) Pp. 14. The Persistence of the Common Law. By Professor Hamish Ross Gray. (Inaugural Lecture delivered in the University of Birmingham on 19th March 1970.) Pp. 21. (Birmingham: The University, 1970.) [128]

The Intensification of Beef and Sheep Production. Edited by S. B. Heath and M. F. Seabrook. Pp. 98+3 plates. (Reading: Reading University Agricultural Club, 1970.) 5s 6d. [138]

Ministry of Technology. Torry Research Station. The National Collection of Industrial Bacteria. Catalogue of Strains, 2nd Supplement. Second edition. Pp. 37. (Edinburgh and London: HMSO, 1970.) 4s (20p) net. [138]

Building Research Station Digest. No. 119: The Assessment of Wind Loads. Pp. 12. No. 120: Corrosion-resistant Floors in Industrial Buildings. Pp. 8. (London: HMSO, 1970.) 9d (4p) net each. [138]

Building Research Station. Current Paper 17/70: A Wind-pressure Transducer. By J. R. Mayne. Pp. 3. (Reprinted from *Journal of Physics E: Scientific Instruments* 1970, Vol. 3.) (Garston, Watford: Building Research Station, 1970.) [138]

Building Research Station. Current Paper 21/70: Building Control in Switzerland. By Evelyn Cinula. Pp. 10. (Garston, Watford: Building Research Station, 1970.) [148]

Other Countries

Republique Francaise. Centre National pour l'Exploitation des Océans—Rapport Annuel 1969. Pp. 52. (Paris: Centre National pour l'Exploitation des Océans, 1970.) [108]

FAO/WHO/OIE. Animal Health Yearbook/Annuaire de la Santé Animale/Anuario de Sanidad Animal, 1969. Pp. vi+330. (Rome: FAO; London: HMSO, 1970.) 36s; \$4.50; 22.50 Fr. francs. [108]

International Indian Ocean Expedition. Plankton Atlas, Vol. II, Fascicle 2: Distribution of Fish Eggs and Larvae in the Indian Ocean. Pp. 5+10 maps. (Cochin: The Indian Ocean Biological Centre; Panaji, Goa: National Institute of Oceanography, 1970.) [108]

US Department of the Interior: Geological Survey. Techniques of Water-Resources Investigations of the United States Geological Survey. Book 3, Chapter C1: Fluvial Sediment Concepts. Pp. vii+55. \$0.65. Book 7, Chapter C1: A Digital Model for Aquifer Evaluation. By George F. Pinder. Pp. v+18. \$0.35. Bulletin 1272-C: Fluorite Deposits of the Quinn Canyon Range, Nevada. By C. L. Sainsbury and F. J. Kleinhampl. Pp. iv+22. Bulletin 1316: Bibliography on the Geology and Resources of Vanadium to 1968. By R. P. Fischer and James P. Ohl. Pp. xxxii+168. \$1. Water-Supply Paper 1869-E: Response of Gas-Purges Manometers to Oscillations in Water Level. By J. R. Beck and C. R. Goodwin. Pp. iv+24. \$0.20. Professional Paper 562-E: Fluorescent Sand as a Tracer of Fluvial Sediment. By Vance C. Kennedy and Dorothy L. Kouba. Pp. iii+13. \$0.30. (Washington, DC: Government Printing Office, 1970.) [128]

Publications of the United States Naval Observatory. Second Series, Vol. XVIII, Part V: Photovisual Magnitude Differences of Double Stars. By K. Aa. Strand. Pp. 31. Vol. XVIII, Part VII: Photographic Measures of Double Stars. By V. V. Kallarakal, I. W. Lindenblad, F. J. Josties, R. K. Riddle, M. Liranian, B. F. Mintz and A. P. Klugh. Pp. 128. Astronomical Papers prepared for the use of the American Ephemeris and Nautical Almanac. Vol. XX, Part II: Heliocentric Coordinates of Ceres, Pallas, Juno, Vesta, 1928-2000. Pp. 137-309. \$2. (Washington, DC: Government Printing Office, 1969.) [128]

New Zealand. Report of the National Research Advisory Council for the year ended 31 March 1970. Pp. 32. (Wellington: Government Printer, 1970.) \$0.20. [128]

Annals of the South African Museum. Vol. 55, Part 3: The Distribution of the Fishes of the Family Clinidae in Southern Africa. By Mary-Louise Penrith. Pp. 135-150. (Cape Town: South African Museum, 1970.) [128]

Smithsonian Contributions to Anthropology. No. 13: Sandpaintings of the Navaho Shootingway and The Walcott Collection. By Leland C. Wyman. Pp. xii+102 (44 plates). (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) [128]

Hydrologicka a Meteorologicka Sluzba Ceskoslovenske Socialisticke Republiky/Hydrological and Meteorological Service of the Czechoslovak Republic—Descriptive Brochure. Pp. 53. Podnebi: Ceskoslovenske Socialisticke Republiky. Souborna Studie. Vydava Hydrometeorologicky Ustav. Redital Josef Zitek. Pp. 356. (Praha-Smichov: Hydrometeorologicky Ustav, 1969 and 1970.) [128]

Development of a National Information System for Physics. By Kenneth D. Carroll. Pp. 9. (New York: American Institute of Physics, 1970.) [138]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 67-66: Geology of Mount Stewart—Souris Map-Area, Prince Edward Island. By G. H. Crowl. Pp. v+26. \$2. Paper 69-42: Precambrian Geology of Hecla-Carroll Lake Map-Area, Manitoba-Ontario. By I. F. Ermanovics. Pp. iv+33. \$2. Paper 69-48: Rigolet and Groswater Bay Map-Areas, Newfoundland (Labrador). By I. M. Stevenson. Pp. iv+24. \$1.50. (Ottawa: Queen's Printer, 1970.) [138]

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 139, No. 7 (June 12, 1970): The Galaxiid Fishes of New Zealand. By R. M. McDowall. Pp. 341-431. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1970.) [138]

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Way Forward, No Way Back

WHAT is happening in the developing countries? And what can be done to make the transition between the past and future at once smoother and more civilized? Two conferences in the past few days have raised important and contrasting issues. At Aspen (see page 1083) it seems to have been broadly agreed by a conference composed largely, although not exclusively, of liberally minded people from developed countries that one of the most urgent problems is the control of population growth. At the British Association in Durham, however, Professor T. A. I. Grillo of Ibadan, Nigeria, has roundly stated that he doubts whether Africa "has a population problem" and even hints that the whole issue may be a plot in which the advanced countries of the world lead the developing countries up the garden path (see page 1081). At Aspen, people also seem concerned that no amount of economic assistance to the developing nations should prevent them from following their own individual paths to prosperity—or even something other than prosperity if they choose; the doctrine of the multi-valued society (whatever that may mean) is to the fore. In Durham, by contrast, Professor Grillo stoutly defends the decision of the Nigerian government to follow substantially the pattern of university development now classical in the United States, the United Kingdom and Western Europe, and even goes one better by insisting that Nigerian medical schools will "not fall into the trap" which seems to him to be represented by attempts at the reform of the medical curriculum now being made in the United States and Britain. On the face of things, of course, Professor Grillo should know best, and there is, in any case, a profound lesson in his declaration that the best way of helping other people is first to find out what other people want. Yet there is also much in what the Aspen people say.

To begin with, Nigeria is exceptional. It has a population (60 million) larger than that of Britain and a strong tradition of university teaching. In the old days of the British Commonwealth (within which Nigeria was always a protectorate, a slightly different entity from a colony), British civil servants always considered their work there to be superlative. Great things were expected. Paradoxically, the civil war (now mercifully at an end) has demonstrated that these brave hopes were justified. The country, after all, is still intact after a domestic tragedy which is by all accounts comparable with that which ravaged the United States a century ago. Moreover, Nigerians of all parties, possibly with the exception of those from the north, will probably in the long run be glad that the civil war was eventually resolved without sub-

stantial assistance from outside, East or West. To be sure, it would be folly to generalize from this example to the proposition that civil war is a way of becoming strong—most countries would not have survived what Nigeria has been through. Most countries even at comparable stages of economic and industrial development, lacking as they do the oil that has made Nigeria viable, would have found the experience to be fatal. To say this is not in any sense to diminish the importance of what has happened in Nigeria, but merely to suggest that the example is not entirely representative. Yet there is something in Professor Grillo's demeanour that deserves to be noticed outside Nigeria.

This, it seems, is what the people at Aspen have acknowledged with their declaration that ways must be found of allowing developing nations to develop in their own ways. The truth may be that developing nations prefer—or find it convenient—to follow the classical path of development in the industrialized parts of Western Europe, but that is not an external constraint but an act of will. For, as it turns out, the developing nations are much more in charge of their own destinies than they were a decade ago. In some places, economic and social difficulties have become worse, but the benefits of a decade or more of independence and of educational development have begun to accrue. With this development has come such a diversity within what used quite inappropriately to be called the underdeveloped world that generalizations are almost always offensive as well as wrong. This is a transition to be reckoned with.

So does it follow that the developing nations can now be left to fend for themselves? To many people in industrialized societies, this is a tempting speculation. It is also dangerous on at least two counts, one positive and familiar—one a product of the new confidence of the developing nations.

The economic gap between the rich and poor countries is as much a source of danger now as ever, and for a variety of reasons. The old notion that the envy of the developing nations for their richer neighbours might lead to conflict of the kind which set the Huns against the Roman Empire is far-fetched, but there is no doubt that economic disparities breed international tension over issues such as tariffs, nationalization and currency control. There are also powerful arguments related to the view that everybody would be richer in a larger market, which boils down to saying that the economic self-interest of industrialized societies requires that the process of development should be hastened. In this sense, foreign aid should be regarded not as charity but as long-term

investment in prosperity for nations of all kinds, at present either rich or poor. By the same token, there should be a general welcome for the prospect of a much more easy-going interchange of people and ideas between the developing and the developed nations. Just as a larger market is richer, so a larger intellectual community is more powerful and self-stimulating. In short, the case for foreign aid and technical assistance has not diminished.

But why, then, do the Aspen people and Professor Grillo differ on the importance of the population growth in the developing nations? To be sure, Latin America and South-east Asia are the population blackspots. By contrast, such imbalance as there may be between people and food in Africa is as much a consequence of ignorance as poor agriculture. That said, however, the belief that Africa can snap its fingers at the growth of population is mistaken, for it is now, as ever, a real problem to balance present growth of population and prosperity. In many advanced societies, it has become common for individual families to make this kind of comparison for themselves, and one consequence has no doubt been the comparatively rapid fluctuation in recent years of the birth rate in countries such as the United States. Even if countries like Nigeria had secretly chosen as long-term goals the attainment of a very large population, it might nevertheless be sensible to aim in the immediate future at the comparative economic

prosperity (and the declining death rate that would result) that a restricted birth rate would bring. That, in short, is the case for a restriction of the rate of growth of population. If the advanced nations were malevolently disposed towards the rest of the world, the chances are that they would preach procreation.

If malevolence is absent, however, indifference is rife. One of the fearful developments in the past few years is the preoccupation of the developed nations with their own affairs. The American involvement in South-east Asia is a typical example—military assistance, so to speak, has replaced technical assistance. But the problems of American society are also distracting, with the result that there is very little hope that Congress will easily be persuaded, before 1972, that the purse-strings should be loosened. Indeed, the way things are going in Washington, Congress may yet devise a means of taking away with one hand what it has given with the other by erecting tariffs to keep from the United States market goods which have been manufactured in developing countries. This will be cruel and absurd. And unhappily there is at present very little sign that the nations of Western Europe are ready to fill the gap. Here again, preoccupations with the European Economic Community or with the relationship of East and West are distracting. On the whole, the balance lies towards gloom, not optimism.

Over the Hill into the Sunset

THE British Association continues to defy the prophecies of those who expect that it will soon die, but only by the narrowest of margins. The meeting this year at Durham has been a considerable success, at least as far as the composition of the meetings goes, but there is no mistaking the signs of infirmity in the ageing limbs. The ructions this year have taken different forms, but the causes of discontent remain what they have been for several years. The organization is poor. The programme, like the curate's egg, is good only in parts. Certainly it is not firmly planned, the efforts of the new planning committee notwithstanding. And the hierarchy is top-heavy, too slow to move with events, now timed with hour-glasses, not calendars. As a result, the association for all its splendid past is much less influential than it could be. Whom the gods will destroy, they first make effete—this seems to be the moral.

The immediate causes for alarm, open secrets every one, add up to make a sombre picture. There is the threat of a severe financial crisis. Half of the association's regular income of £50,000 comes from government departments as subventions for the worthy work

of bringing the message of science to young people and for introducing many of them to technology. There is too little elasticity in the remainder for the association to be able to accommodate inflation. The crying need is for an independent source of income, but it appears instead to have responded to a serious situation by economizing on staff in a manner unlikely to command the affection of those still on the payroll. There has also been, at Durham, the constant skirmishing between chairmen of scientific meetings and the eloquent and sometimes articulate members of the British Society for Social Responsibility in Science: impending law-suits apart, there is a case for thinking that the BSSRS had the better of the encounter. Certainly the dissidents made the meetings lively. Predictably, perhaps, the association responded as would an elephant or—more worrying—a dinosaur.

So why not look the other way and let the breath ooze slowly from the collapsing frame? There are several reasons for taking care. For one thing, the British Association has its roots in the years at the beginning of the nineteenth century when the Royal Society was moribund for lack of discrimination and

when the young men who laid the foundations of Victorian science were crying out for a forum in which to talk to each other. The association's achievement has been great. It has been in turn a learned society, a means of influencing policy on scientific administration and research, a coliseum for the confrontation of ideas (as with the row between Huxley and Wilberforce on Darwinism or the puzzling towards the end of the century about the relationship between electromagnetism and relativity), and a device for the enlightenment of many people who wish to learn about science or merely keep up to date as well as an active source of pleasure for those who attend the annual meetings. More recently, there have been cheerful if shoestring attempts to interest young people in these kinds of things. Maybe the role of the association as a place in which new results in scientific research are brought before the scientific profession, is no longer necessary, but there is no mistaking the honest goodwill that serves still to keep the association's head just above the water. It would, of course, be folly to waste such an asset, especially now that there has emerged more clearly than ever a need for level-headed discussions of social issues to which the supposedly socially responsible scientists have this past week somewhat irresponsibly drawn attention.

So what is to be done? That change should turn out to be desirable in changing circumstances should not be a surprise. The modern world abounds with illustrations of how hallowed institutions must either change with the times or be overtaken. As luck will have it, and if only the association would see it, the changes required of this cumbersome framework are a good deal less drastic than many institutions have been compelled to undergo. And the overriding need is to have a clear strategy for the years ahead. What is the association trying to accomplish? The enlightenment of a thousand or so members at the annual meeting, worthy cause though it may be, is not a sufficient excuse for continuing to exist. Even the work with young people is not a sufficient justification—quite separate organizations might accomplish the same ends. If the association is sensitive at all to its great traditions, it will continue into the present the influence which it could exert a century ago on the way in which science as a whole is accepted into public life. There is no shortage of important matters to be dealt with—the world is full of them.

The directions in which change could be sought within the British Association are also, as luck will have it, plain for all to see—if they have the will. Although money does not grow on trees, there is no reason why the association should not add to its activities in ways that will allow it to prosper. Other such bodies publish books, undertake research and other investigations of public importance, and sometimes even find themselves able to allow members to attend annual meetings and the like without levying steep charges on them. With ingenuity, wit and a little energy, it should be possible for the association to put its books in order without having to pass

around the begging bowl in the way which seems at present to be the closest approximation to financial management.

A good deal of the necessary reforms would be more easily accomplished if the organization of the association were not a kind of fossil record of its history. Over the years, hierarchies of committees have grown up in such a way that nobody can tell where power lies. With the convention that the president should be a luminary plucked from outside—a Nobel prize-winner if possible, but otherwise somebody on whom public honour has already fallen—the permanent general secretary of the association should be the one not merely to keep the association running smoothly but to hammer out policy and to present the association's case to the outside world. But is this man intended to be a leader or merely a servant of the bureaucracy? The association has never been clear in its mind on this, with the result that there is a small group of general officers whose function seems to be to make sure that the general secretary toes some imaginary line in the conduct of the association's business. The general conduct of policy, on the other hand, is supposed to reside with the council, much larger than efficiency requires and, in any case, less able than it should be on account of its part-time character to keep a constant watch on the association's business. Furthermore, a skilled president can always hope to keep at least one step ahead of his council, but there are always dangers that even he might be overruled by either the body known as the general committee or, failing that, by the General Assembly—the committee of the whole, as the Americans would say. If this division and scattering of power were not a sufficient offence against the principles of good management, however, there is a further complication. The sections of the association normally responsible for the specialist parts of the scientific programme have ill-defined but substantial degrees of autonomy (which is one reason why the scientific programme never quite hangs together). Nobody knows how the relationship between the sections and the rest of the association is determined, but the relationship is beyond doubt archaic. One of the most urgent needs is that this complicated organization should be simplified. On the face of things, it is hard to see how the work can be done entirely from within the association.

In much the same way, responsibility for the routine work of the association is divided between the full-time professionals at head office and the small arms of volunteers throughout the length and breadth of Britain who spend their precious time addressing envelopes to each other. Although a simpler organization could drastically reduce the waste of energy involved, greater professionalism in the conduct of the association's business would probably make necessary an increase of staff and not the reduction on which the association has somewhat precipitately embarked.

Is there, then, a chance that all this can be mended from within? It is at least a sign of grace that the association has decided at long last to set up an inquiry

into its own affairs. High time is what people will say. But everything depends on how the inquiry is carried out. How much in the thrall of the existing hierarchy of committees and officials will it be? There is the strongest case for asking that the chairman of the inquiry should be an independent well-wisher from outside the immediate circle in which power is at present distributed. It is also essential that the inquiry should be quick. If by this time next year the association is not in better shape, there is a serious risk that it will bleed to death.

100 Years Ago



And looking back through the prodigious vista of the past, I find no record of the commencement of life, and therefore I am devoid of any means of forming a definite conclusion as to the conditions of its appearance. Belief, in the scientific sense of the word, is a serious matter, and needs strong foundations. To say, therefore, in the admitted absence of evidence, that I have any belief as to the mode in which the existing forms of life have originated, would be using words in a wrong sense. But expectation is permissible where belief is not; and if it were given me to look beyond the abyss of geologically recorded time to the still more remote period when the earth was passing through physical and chemical conditions, which it can no more see again than a man can recall his infancy, I should expect to be a witness of the evolution of living protoplasm from not living matter. I should expect to see it appear under forms of great simplicity, endowed, like existing fungi, with the power of determining the formation of new protoplasm from such matters as ammonium carbonates, oxalates and tartrates, alkaline and earthy phosphates, and water, without the aid of light. That is the expectation to which analogical reasoning leads me; but I beg you once more to recollect that I have no right to call my opinion anything but an act of philosophical faith.

As regards the second problem offered to us by Redi, whether Xenogenesis obtains, side by side with Homogenesis; whether, that is, there exist not only the ordinary living things, giving rise to offspring which run through the same cycle as themselves, but also others, producing offspring which are of a totally different character from themselves, the researches of two centuries have led to a different result. That the grubs found in galls are no product of the plants on which the galls grow, but are the result of the introduction of the eggs of insects into the substance of these plants, was made out by Vallisnieri, Reaumur, and others, before the end of the first half of the eighteenth century. The tapeworms, bladderworms, and flukes continued to be a stronghold of the advocates of Xenogenesis for a much longer period. Indeed, it is only within the last thirty years that the splendid patience of Von Siebold, Van Beneden, Leuckart, Küchenmeister, and other helminthologists, has succeeded in tracing every such parasite, often through the strangest wanderings and metamorphoses, to an egg derived from a parent, actually or potentially like itself; and the tendency of inquiries elsewhere has all been in the same direction. A plant may throw off bulbs, but these, sooner or later, give rise to seeds or spores, which develop into the original form. A polype may give rise to Medusae, or a pluteus to an Echinoderm, but the Medusa and the Echinoderm give rise to eggs which produce polypes or plutei, and they are therefore only stages in the cycle of life of the species.

An extract from the opening address of T. H. Huxley to the 1870 meeting of the British Association, held at Liverpool. From Nature, 2, 404, September 15, 1870.

OLD WORLD

BLACK ARROW

Crisis of Confidence

THE failure of Black Arrow to launch a satellite last week has placed the Ministry of Technology in an embarrassing position. Should last week's firing be repeated, making further inroads into the stock of rockets on order, or should the programme go ahead as planned, in which case a £2 million satellite will be risked in a firing early next summer? The heart-searching behind this decision is one of the penalties to set against the shoe-string budget on which the Black Arrow is based—£3 million this year, gradually rising to £5 million during the next two years. Unsuccessful firings are now rapidly eroding the programme. In June last year the first Black Arrow with a dummy third stage went off course after fifty seconds of flight because of a fault in the control system and had to be destroyed. That meant that instead of the first orbital attempt in March this year there had to be a repeat of the earlier firing. Now the postponed orbital attempt has failed, apparently because of a fault in the pressurization of the second stage oxidant tank.

The chronology of the firing was as follows. First, a fault at the Gove tracking station, northern Australia, caused a postponement on September 1 in the last few minutes of the countdown. Gove is an ELDO tracking station that was due to be closed down now that launchings of the Europa rocket are to be from French Guiana, but was kept on for the Black Arrow launch. Although tracking by Gove is not essential, it gives a useful cross-check. The fault was in the computer that steers the aerial, and was discovered after the nominal flight path was fed in just before launch. Because the computer could not be put right within the duration of the launch window, the firing was postponed to the following day, and then delayed a further 2½ hours because of weather conditions. The faulty pressurization caused the second stage engines to stop thirteen seconds early, so that the spent stage crashed only 800 miles north of Woomera, 1,400 miles short of the expected impact point. Although the third stage subsequently seems to have worked perfectly, too much velocity was lost for the satellite to go into orbit.

To make matters worse, both failures of Black Arrow were due to faults in what were believed to be some of the soundest parts of the rocket. Basically a nitrogen bottle and a valve, the pressurization was thought to be simple and reliable. It now looks as if the sealing up of the highly successful Black Knight two-stage research rocket has not gone as well as the Ministry of Technology must have hoped when it earmarked only three Black Arrows for development firings. These are now spent, and there is no possibility of another development firing before next summer when the X3 satellite is due to be launched, chiefly to test new satellite instrumentation. It is too early yet to say what effect the failure will have on the ministry's hopes of selling Black Arrow rockets to ESRO, although they must be extracting some crumbs of comfort from the news that their competitor, the French Diamant rocket, is said to be running into trouble with excessive vibrations imposed on the payload.

What a sad start for Mr A. Goodson, who took over from Mr R. H. W. Bullock as head of the Space Division at the Ministry of Technology on the morning of Black Arrow's failure.

All to Go at Gove

THE down-at-heel air that surrounds Britain's Black Arrow space programme is well illustrated by the news that the ELDO tracking station at Gove, Australia (where a computer failure caused the first postponement of last week's space shot) is up for auction. The sale will take place on October 20. All the station's equipment is to be auctioned off including presumably the errant computer and tracking dish that figured in last week's Black Arrow launch. For the Black Arrow firing last week the Ministry of Technology borrowed Gove from ELDO. Its function was to monitor the performance of the third (apogee motor) stage of the launch, a task that should in future be carried out by an onboard inertial apparatus on flight test for the first time last week. Some of the ELDO equipment in Australia is to be shipped to the Kourou range in French Guiana, where the remaining ELDO launchings will take place, but all of the Gove material is for disposal at the October auction. ELDO, chronically short of funds, hopes in this way to recoup some of its capital outlay. The sale is being widely canvassed among interested parties in the area—presumably the Japanese, the Americans and the various military establishments that make use of Australia. By a deft piece of public relations the announcement of the Gove auction occurred the same day as the postponement of the Black Arrow launch due to the failure in the Gove equipment.

DEFENCE

No Rest in Arms Race

WHILE the preliminary rounds of the Strategic Arms Limitation Talks (SALT) were going on earlier this year, the nuclear arms race showed little signs of slowing down. The Soviet Union, during the twelve months ending on June 30, 1970, added 250 new land-based and 120 sea-based intercontinental ballistic missiles to its arsenal, thereby putting it ahead of the United States in terms of sheer numbers of land-based missiles. But the United States continued to develop the Minuteman-3 and Poseidon multiple independent re-entry vehicles (MIRV) and a decision to expand the "safeguard" antiballistic missile system has just been taken by Congress. These developments, mapped by the Institute of Strategic Studies, provide ample proof that the policy of assured destruction capacity which has guided the defence policies of the United

States and the Soviet Union during the 1960s has continued into the 1970s, SALT talks notwithstanding, and these two powers alone are planning to spend \$114,778 million on their defences this year (*The Military Balance 1970-71*, ISS, 13s).

While the nuclear merry-go-round continues at this alarming rate, and the two chief military powers seem reluctant to step off, other powers are anxious to join in. The launching of China's first satellite in April this year inevitably increased speculation that Chinese missiles may soon be capable of hitting cities in the United States but, according to the Institute of Strategic Studies, there is still no evidence that the country has yet developed operational missiles, and its ability to deliver nuclear weapons is still limited to early bombers. Nevertheless, the long-term threat of that development seems to have provided a powerful lever for getting the appropriations for the safeguard system accepted by Congress.

As far as conventional forces are concerned, the Institute of Strategic Studies points out that the Warsaw Pact countries have some 11,000 more tanks and 2,000 more tactical aircraft in Europe than the NATO countries have, and that although the West is still superior at sea "Soviet fleets are now able to challenge at every level of military or politico-military activity". But it is in the Middle East that the chief economic effects of military build-up are being felt. Israel has planned this year to spend a quarter of its gross national product on defence, or a staggering \$400 for every Israeli citizen. The only other nation which comes close to spending that much is the United States, which reckons to spend \$393 a head this year, while Britain, for example, should spend about \$100. Egypt on the other hand, is planning to spend slightly more in absolute terms than Israel, but this amounts to about 13 per cent of its GNP.

SEAL DEATHS

From Natural Causes

FORTY or so grey seal pups which were found dead or dying on the Cornish coast last autumn were suffering neither from pollution, nor from an epidemic, but from starvation after premature separation from their mothers. The deaths, in other words, were part of the normal juvenile mortality of the local seal population, according to Mr W. N. Bonner of the Seals Research Unit at Lowestoft, who has been conducting an investigation for the Natural Environment Research Council (*NERC publ. series C, no. 1, 1970*). On average some 60 per cent of all pups die in their first year, and this figure is likely to vary widely in stormy weather or crowded conditions, when pups can easily lose their mothers during the crucial three-week suckling period.

In view of the widespread publicity that the deaths received, and the ensuing speculation about pollution which blew up because of the Ministry of Defence experimental establishment at Nancekuke (Cornwall), eleven of the dead seals received post-mortem examinations. In all cases the primary cause of death was malnutrition; four of the seals had complications such as pneumonia or septicaemia, but the rest had simply starved. No startling amounts of contaminant were found in tissue samples, and even though the seals' blubber contained more polychlorinated biphenyls than expected, the concentration was well below that found

Table 1

Table 1. GROWTH OF INTERCONTINENTAL BALLISTIC MISSILE (ICBM) AND SUBMARINE LAUNCHED BALLISTIC MISSILE (SLBM) STRENGTHS 1960-1970.

		1960	1962	1964	1966	1967	1968	1969	1970
USA	ICBM	18	294	834	904	1,054	1,054	1,054	1,054
	SLBM	32	144	416	592	656	656	656	656
USSR	ICBM	35	75	200	300	460	800	1,050	1,300
	SLBM	—	some	120	125	130	130	160	250

in routine analyses of East Anglian seals. As far as the effluent from Nancekuke is concerned, Mr Bonner finds that the standard of treatment is high enough to leave the Ministry of Defence wholly blameless.

Why then did so many seals die last year? One possibility is that the initial publicity encouraged more people than usual to report the dead seals they had seen—in most years there are eight or nine reports of pups in difficulties, but dead seals are usually ignored because nothing can be done for them. Some pups may have been driven to Cornwall from the colonies in Pembrokeshire during periods of strong winds in early November, and bad weather would in any case bring about heavy mortality among the seals which breed on the inhospitable Cornish coast. Extraneous factors thus seem unnecessary for an adequate explanation, and natural causes can also account for lesions seen on the necks of four adult seals at the same time, because seals often fight during the breeding season and receive wounds which can become infected.

Only about sixty to seventy-five pups are born in Cornwall each year, so the mortality rate seems to have been very high indeed. A tour of the breeding localities in December, however, revealed at least six young seals, of which all but one appeared perfectly healthy, and allowing for those which would have been hidden away in caves or other inaccessible parts of the coast a fair survival of pups seems likely.

EDUCATION

Soviet Students Better Prepared

from our Soviet Correspondent

THE new academic year always receives massive coverage in the Soviet press. This year, the last of the current five-year plan, the opening of schools and universities has been even more extensively reported than usual. The implementation of the resolutions of the twenty-third party congress must be discussed and tentative proposals made for the forthcoming twenty-fourth congress.

The expansion of higher education was one of the chief policies of the twenty-third congress. According to the Soviet Minister of Education, Comrade V. P. Elyutin, during the past five years 48 new higher education establishments have been opened in the Soviet Union, including eight universities, fifteen technical-engineering institutes, an institute of electronic technology (in Moscow) and several other specialized institutes and polytechnics. Special emphasis is placed on the training of young specialists for the new professions, such as automation and cybernetics, or in those subjects, such as geology, which have a particular importance in Soviet planning policies.

Recent educational directives and laws have been aimed at providing higher education for young people with a good work record in factory or collective farm, and for ex-servicemen. Special training schools have been established to prepare them for university entrance, and accordingly they are well-represented in the 900,000 new students entering Soviet universities and institutes of higher education. (Of this 900,000, half a million are full time students, and the remainder will be attending part-time and evening courses.) Presumably as a result of the establishing of these training schools, the Minister says that the general educational level of the new students has risen considerably.

FOUNDATIONS

Mr Young's Last Year

THE Nuffield Foundation this week published its last annual report under the directorship of Mr Brian Young, who takes up his appointment next month as Director-General of the Independent Television Authority. Mr Young's departure, after six years with the Nuffield Foundation, comes at the end of a year in which the foundation awarded nearly 100 new research grants worth more than £1.3 million. Most of the grants were awarded in accordance with policies established during the past few years.

In scientific research, for example, the emphasis was on biology—£250,476 was allocated to biological research compared with £16,946 to other scientific research. The foundation defends such a bias on the grounds that "the physical sciences, and technology, are already more lavishly provided for, yet it may well be that the greater needs—not only in terms of money but with the over-all benefit to society in mind—may lie in biology".

Again following the pattern set by previous years, the foundation's educational programme was given the largest share of the foundation's grants in 1969, but attention is now being directed chiefly towards higher education. This in part reflects the success of the Nuffield-sponsored schools curriculum projects during the 1960s, which have led to a change in the nature of the university and college student intake. A grant of £80,000 has been awarded to five universities—Bath, Birmingham, Glasgow, London (Chelsea and Queen Elizabeth Colleges) and Sussex—to collaborate on a project designed to produce new teaching materials for undergraduates in the biological sciences. Two grants were also awarded in 1969 for research in the field of teacher training, one of which is aimed to prepare science teachers for the new types of secondary school curricula—many of them associated with the foundation's own Science Teaching Project.

As far as the social sciences are concerned, the foundation continued last year to back people rather than programmes. For example, Professor A. J. Brown of the University of Leeds was given a grant of £8,250 for work on regional economics, Professor Max Gluckman of the University of Manchester was given a grant to relieve him of the commitments of regular teaching and administration so that he can devote more time to studies of comparative politics and law, and Professor Sir Karl Popper has been given a grant to carry on with his studies after his retirement from the chair of logic and scientific method at the London School of Economics.

Table 1. NUFFIELD FOUNDATION GRANT ALLOCATIONS 1969

	Total allocations	Allocations 1969
Science		
Biological research	2,967,397	250,476
Other scientific research	1,690,624	16,694
Medicine	4,671,890	113,493
Social research	3,562,011	200,516
Education	4,372,137	303,787
Care of aged	2,058,636	115,339
Commonwealth	3,857,393	83,750
Fellowships and scholarships	3,114,075	186,430
Recoverable grants	270,400	32,500
Total	26,564,563	1,303,237

EDUCATION

Computers for the Million

ALL undergraduates, and not just those specializing in the sciences, should be taught how to use computers. This is the conclusion of a joint working party set up by the University Grants Committee and the Computer Board as much as anything because of concern about the widespread lack of awareness of the potential of computers (*Teaching Computing in Universities*, HMSO, 2s 6d). Although their blanket recommendation of programming experience for everybody may cause dismay among arts and social science students, undergraduate scientists will be glad that the inevitable introduction to computing occurs sooner rather than later. Too much time is lost by people having to start from scratch at postgraduate level. The justification for including non-scientists is that graduates from all disciplines take up posts in industry and government, where an understanding of computers is becoming increasingly important. Although it would obviously be preferable if everybody learnt the rudiments of computing at school, the working party, under Professor G. A. Burnard (University of Essex), is working on the principle of better late than never.

At present the chief obstacle to the spread of computer education at undergraduate level is a paper one. The machines supplied by the Computer Board, the chief source of computers for the universities, are limited by the board's terms of reference to use in research only. Several directors of computing laboratories told the working party that they felt unable to increase the amount of teaching because of the board's terms. At the working party's recommendation the government has now agreed to extend the terms of reference to include the supply of computers for teaching. In a foreword to the report Mr Kenneth Berrill, chairman of the UGC, and Professor Brian Flowers, until recently chairman of the Computer Board, say that the extra hardware required between now and 1973 will mean an additional expenditure by the board of only about £1 million, and that a start can still be made on carrying out the working party's recommendations. The report says that a realistic time for the introduction of elementary courses would be the session 1972-73.

Teaching in computing at undergraduate level is at present patchy and thin, the report says, and at some universities non-existent. The report suggests that an introductory course should be run usually in a student's first year, preferably with the students having direct access to a computer through on-line consoles, although the report recognizes that, to begin with, most universities will have to settle for indirect access using card or paper tape inputs. This will mean developing compilers that allow a computer to handle a batch of simple programs that might be produced by a class of students more quickly than if the computer went through the normal operating procedure with each separate program.

It would be best, the report says, if the elementary courses were not taught by the staff of each university's computer laboratory but by the staff of the students' department. Departmental staff will be better able to stimulate the students with relevant applications of computing, but demonstrators should help the students with their programs.

ART COLLEGES

Art without Design

THE general malaise of art education, which has only been brought to public attention by the bitter and protracted disputes at Hornsey and Guildford colleges, is unlikely to be greatly alleviated by the long awaited report of the Coldstream Committee. The committee, under the chairmanship of Sir William Coldstream, has been looking into the whole structure of education in art and design, and it has published its recommendations this week (*The Structure of Art and Design Education*, HMSO, 7s).

The committee suggests, but without much discussion of the rationale behind the suggestion, that the present Diploma in Art and Design (Dip AD) should be substantially modified. Some of the design courses should be taught separately as four-year sandwich courses, with up to one year spent in industry, while the remaining courses should be flexibly grouped into four chief studies—fine art, graphic design, three-dimensional design and textiles/fashion. The committee lays considerable emphasis on the flexibility of the groupings, and suggests that students should be able to pursue a broader range of studies which cross or overlap the boundaries of the groupings. The sandwich courses, on the other hand, should have a "substantial specialized technological content which can best be studied in close association with the relevant industry or profession". Students should be able to transfer from the sandwich course to the Dip AD or vice versa only in exceptional circumstances.

The committee also suggests that students should be able to enter the sandwich courses after a good general education of sixth form standard, while those wishing to take the three-year Dip AD course must still take the one-year foundation course except in special circumstances. But the most grave and pressing problem at present facing the potential art student is that many more students leave the foundation courses in search of a diploma course than the diploma colleges can accommodate. It is this huge demand for places that creates much of the frustration and discontent in art education. What the committee proposes is essentially that the number of places in foundation courses should be limited: "some form of central control of foundation courses is necessary so that the number of people being prepared for diploma courses and the number of diploma places available relate more closely to each other", and so the bottleneck will be shifted but not removed.

How does the development of the polytechnics, and the general expansion of further education affect the committee's recommendations? Unfortunately, the recommendations seem to have been made without much regard to—or at least without much discussion of—the rest of the education sector. To be sure, the report points out that there are some considerations which have not been included because the committee was anxious that its recommendations should be made as soon as possible, but a discussion of the part to be played by the art colleges in the polytechnics and some consideration of the interactions between art colleges and, for example, colleges of education seems a striking omission.

British Association

Who is Responsible?

from our Special Correspondent

Durham, September 8

THE luckless British Association, assailed as it is by financial troubles from within, is now being sued by two members of the British Society for Social Responsibility in Science on the grounds that the secretary, Dr Henry Turner, earlier this year circulated within his organization a statement announcing the intention of the BSSRS to intervene at this year's meeting at Durham in terms which, it is alleged, were defamatory of some members of the society. Earlier this week, the parties alleging injury also put out a statement protesting that the "hierarchy" of the British Association has been unresponsive to the real questions of the day and, for practical purposes, giving up the British Association as an organization beyond redemption.

The activities of the BSSRS—represented here by research students for the most part—have been diligent, amusing but often beside the point. Their activities began at the end of the opening ceremony last week, when several people enacted the perils of nerve gases. There has also been a stream of pamphlets and broadsheets denouncing the supposed irresponsibility of the British Association. Many members of the association were also present at the teach-in last Saturday, when the question for debate was the neutrality (or otherwise) of science.

The most constant reminder of the presence of the BSSRS has been the stream of interventions at the formal proceedings of the sections of which the British Association is composed and—fair play—most of these have been polite if not articulate. But many well-wishers must have been downcast at the factual inaccuracies with which the attacks on what is called the Establishment are larded. (One of the more tricky questions for the students—apparently unanswered—has been posed by the way in which Professor John Ziman, the president of the General Section, has consistently sported a button with the letters SRS for "Social Responsibility in Science".)

The organizers of the meeting, many of whom clearly appreciate the problems that the BSSRS is worried about, may have felt pangs of indignation, for some sections seem to have made an effort to bring social and environmental questions to the fore. Those devoted to sociological and general topics led the way by spending a day discussing the sociology of science. And the botanists, perhaps still smarting under last year's jibes that their subject is dying or dead, heard their president, Professor P. F. Wareing, speculating on how they could help to increase the world's production of food. The geography section devoted a morning to discussion of

population and development in the Middle East and another to the problem of derelict land. Durham, with its 16,000 acres of officially derelict land, is a fitting place for such a discussion, especially in view of the good work done already in reclaiming much of it (page 1082).

The environment, as might have been expected in European Conservation Year, received plenty of attention. Apart from an exhibition on water resources, and various individual contributions throughout the week, four sections, comprising biologists and geographers, combined to mount a large scale symposium concerned with conservation and productivity. For this, speakers from the Nature Conservancy turned out in force.

There was perhaps some justification for the impatience of the SRS group with the general reluctance to discuss political aspects of pollution, population and so on. Much of what was said about the need for conserving wildlife and fisheries seemed to have been heard more than once before. A broader discussion might not have proved too much for BA members, many of whom must be convinced conservationists in any case.

Putting man first, the sociologists spent a morning discussing public participation in planning, and an exhibition called Phoenix '70 illustrated public and private contributions to the social and economic development of the North-East of England. One of the more interesting of a generally uninspiring handful of displays was provided by Durham Constabulary and the local Home Office forensic science laboratory. It is good to know that X-ray crystallography, infrared photography, differential hologram interferometry and emission spectrography have useful applications in the apprehension of criminals. The police were also the subject of a morning's session opened by Professor M. Banton with his presidential address to the sociology section. Medicine was the topic for dissection during a general symposium on new doctors' dilemmas. Dr Henry Miller chaired a day of deliberations on transplantation, treatment of mental and physical abnormality and the misuse of drugs.

Clearly the BA is open to criticism for not giving more prominence to questions of social responsibility, but equally it is unfair to condemn it wholeheartedly, as this year's proceedings have shown. A worthwhile enterprise for the future might be a large scale symposium devoted to the topics that the SRS group were raising at Durham—warfare, the politics of pollution and so on. That at least should not prove too embarrassing.

DEVELOPMENT

Science makes Development Sweet

from our Special Correspondent

A CHEERFUL account of the ways in which scientific institutions are being set up in developing societies was provided at a symposium earlier this week at the British Association's annual meeting at Durham. One striking feature of the proceedings was the forthright rejection of the view that the technology of advanced societies might serve only to corrupt the developing nations by Professor T. Adisanya Ige Grillo, professor of anatomy at Ibadan, Nigeria. The button-wearing doomsdayers seemed for a time abashed to discover that a Nigerian could be as keen on technology as any go-ahead industrialist.

The symposium, organized by Professor John Ziman as part of the proceedings of Section X (for General), was conducted by Lord Blackett, president of the Royal Society and a distinguished recruiter of science in the cause of economic development. The difference between the two categories, he said, is most easily measured by per capita income—\$600 per head on the average for the developed nations and only about \$100 per head for the developing nations. But there has been progress. The developing nations have collectively increased their economic wealth by about 5 per cent a year in the past decade. Yet economic progress remains the goal, and so every scientist must be imbued with the notion that social progress in the developing world must be the goal only next in importance to the avoidance of a nuclear war.

The case for variations of the conventional pattern of science teaching in the developing countries was put by Professor D. G. Osborne of University College, Dar es Salaam. Science graduates, like members of the general population, must be "competent for change", he argued, and must be trained for making administrative decisions with a scientific content as much as for research or even teaching, which argues the need for mixed courses—economics and physics, for example. He asked that textbooks written for students in developing nations should be more fully provided with illustrations relevant to the societies concerned, that more graduates from universities in developing nations should be able to have postgraduate training in other countries and he pushed for innovations such as a scheme to induce developing universities to spawn from within themselves industries suited to regional needs. Among the impediments to scientific education in developing nations are the lack of the kind of general awareness of scientific matters that might be provided by newspapers or other non-professional journals, the linguistic background of the students (not usually English) and cultural restrictions such as the tendency to believe that a person should not dirty his hands.

Dr H. C. Pereira, director of the East Malling Research Station, slew two paper tigers with his declaration that the unwillingness of farmers in the tropics quickly to change their methods has at least the advantage that new crops disastrously susceptible to new diseases are not introduced, and that the merchant banks which operate on the fringes of the cash economy do worthy service for tropical agriculture. Dr Pereira argued strongly for "science on the spot" if practical problems were to be dealt with efficiently.

Nigerians do not easily forget the civil war, only recently at an end, and Professor Grillo several times returned to his statement that the troubles had at least helped Nigeria to recognize its own internal strength. He was scornful in an amusing way about past attempts at aid for developing countries—"you cannot help people without knowing what they want" was one of his themes. He pleaded for "a certain amount of faith" in the capacity of African scientists, however trained, to know what they were about, and claimed that the development of educational institutions in Nigeria at least had now reached the point at which "we are no longer in a position to put the blame on the colonial past . . . if there are mistakes, they are our mistakes". But he thinks it unlikely that Nigeria will ever again establish classical universities—technical institutions are the pattern.

Professor Grillo welcomed the notion that there should be easy exchanges of people between developed countries and Nigeria, but had a sombre warning for the developed nations and especially for Britain about the quality of the people sent to the developing nations as university teachers. With all the links now extant with developed nations, these universities had become places where the developing peoples could make international comparisons and in which the third-rate people were conspicuous.

The development of health services in Nigeria is a centrepiece of the strategy of the National Science Council of Nigeria, and Professor Grillo promised that Nigeria would have a more carefully planned programme of medical research than any in the United States or Britain. He was scornful of the way in which the National Institutes of Health in the United States were persuaded to start a programme of congenital lung diseases in the newborn after the death of one of Mrs Jacqueline Kennedy's children, anxious to say that malnutrition and disease are often just as much consequences of ignorance as of poverty, and yet convinced that health engineering as he called it—the prophylactic management of the environment—is often more important than mere doctoring, nevertheless, Professor Grillo said, Nigeria would in the development of medical schools make sure that intending doctors received a good grounding in the medical sciences.

To many of his audience, Professor Grillo's most surprising declaration was that there is at present no reason why Nigerians should without further thought accept the advice of outsiders that the time has come urgently to reduce the rate of growth of the population. "Is there a population problem in Africa? We doubt it. And all this anxiety about family planning—is it genuine? That's what we have to decide." He went on to quote as evidence in favour of doubt the ease with which "you can get the money for an electron microscope from the aid organizations so long as you say it's for family planning".

The civil war apart, Nigeria (with oil) is luckier than most developing countries. What Professor Grillo had to say reflected also a sense of cheerfulness which seems—at least to judge from the inevitable questions of the students at the back—to be vanishing in the developed world. At one point he was almost on the point of saying that the women's lib would have nothing to ask for in Nigeria, but this statement eventually appeared as "the women of West Nigeria play a very important role".

LAND RECLAMATION

Spoil Heaps into Pastures

from our Special Correspondent

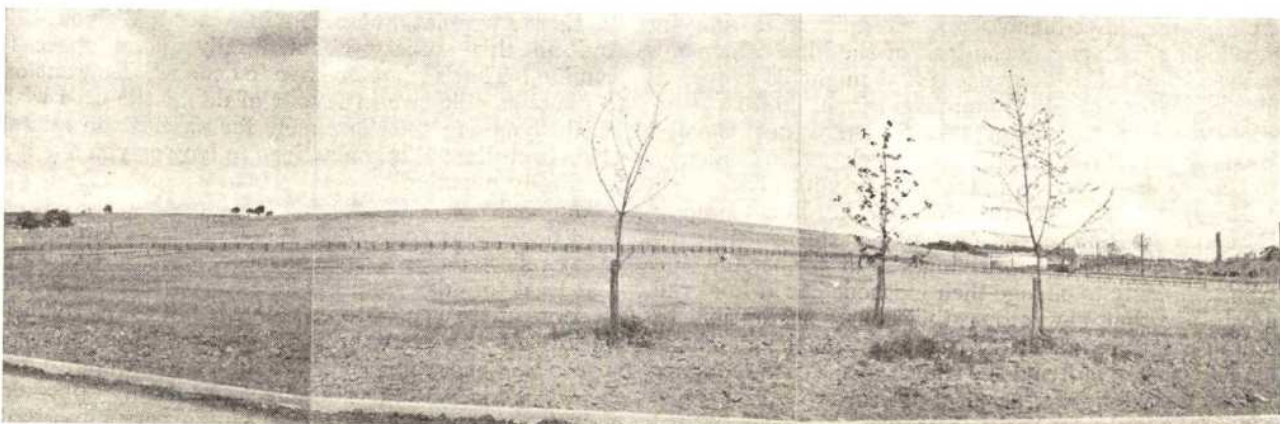
THE hated spoil heaps of Durham are disappearing under a lush growth of grass and trees. The mountains of black shale which remained after the closure of the county's many coal mines are being flattened and planted to convert them to pasture, woodland and recreational areas which defy recognition as the eyesores of the past. Last week some of the pioneering work carried out in Durham gladdened the hearts of members of the British Association. About twenty of them were conducted on a fifty mile tour of about a dozen heaps, reclaimed and being reclaimed, by Dr A. J. Richardson of the Department of Botany, University of Newcastle upon Tyne, and Mr R. Briggs, Durham County Council's Landscape Reclamation Officer.

One of the oldest examples was a small wooded hill which had been a spoil heap planted with trees in 1927 by the county council. In spite of this early start in reclaiming derelict land—long before concern for the environment reached its present status—the council did not swing fully into action until after 1950. Between 1954 and 1960, however, about thirty small heaps were tackled, representing about 700 acres of reclaimed land. After the Local Employment Act of 1960 made government grants available for reclamation (50 per cent of the cost, increased to 85 per cent in 1963), the council set up its reclamation unit. Then it really got to work on the 350,000,000 tons of pit waste deposited in the county during the past 200 years.

The largest scheme so far is in progress near Thinford, where 2.6 million out of 7 million cubic yards of spoil is being spread onto adjacent agricultural land, which, as in many cases, has been purchased by the council. Work began last February, and grass should be growing by the summer of 1971, at an estimated cost of about £278,000.

Another scheme at nearby Sherburn Hill has involved a reduction of nearly 130 feet in an ungainly 200-foot conical heap, which has been merged into an adjacent limestone ridge. Available topsoil is being spread over the new surface ready for seeding with grass. The reclaimed land will be used for grazing, and some trees will be planted. This is costing £105,000. About twelve miles away are forty-five acres of pasture, woodland and open space which were once a 190-foot high spoil heap dominating the town of Willington. Reclamation began with the shifting of about 15 million cubic yard of material in October 1967, and, within a year, grass was growing on the reshaped mound, some of it sown on top soil and some put directly into the shale.

At Reddymoor Colliery, near the town of Crook, the spoil heap is now covered with the grasses *Agrostis tenuis*, *Dactylis glomerata*, *Festuca rubra* and *Festuca ovina*. This heap is part of the University of Newcastle upon Tyne's landscape reclamation project, and various trees and shrubs are being tested for their suitability to such conditions. *Sorbus aucuparia*, the rowan tree, is doing quite well and so is *Rosa rubiginosa*, sweet briar, but *Robinia pseudacacia*, acacia, is not, nor is *Sambucus*, elderberry. Acidity and lack of nitrogen often have to be overcome by a judicious use of lime and fertilizer.



Brancepeth pit heap as it used to dominate Willington (top), and as it is now (bottom) after treatment by Durham County Council.

NEW WORLD

Intellectuals of the World Disunite

by John Maddox

Aspen, Colorado, September 4

ON the face of things, this remote almost alpine valley is either the least appropriate or the most suitable place in which to hold a conference about the relationship between technology and the rest of society. If the problem is the pollution of the environment, the splendid mountains are a sign of how good things can be and the ski-runs a proof that people want not wilderness but blissful recreation. If the issue is economic development, then Aspen is also a good setting, for there are already signs of what the village will be like when great ski lodges have been grafted to the Victorian frontier resort of just a few years ago. Yet in the event, the setting turned out to be unsuitable for the conference on "Technology—social goals and cultural options", organized jointly by the Aspen Institute for Humanistic Studies and the International Association for Cultural Freedom. The proceedings seemed to begin well enough, but in the end the hundred or so participants had little to say except that they had no single view about the place of technology in the modern world. Worse still, this disagreement was conspicuously etched out, for the benefit of local residents and the international press, at a meeting yesterday that will surely be remembered as long as conferences are held as an example of how the nicest and the cleverest people (but perhaps *they* stayed away) can do each other mischief in public. Is the altitude (8,000 feet) responsible? Or the reputation of this valley for being grand? Or is it the simple truth that there can be no simple statement about the relationship of technology and the big wide world?

No report of these confused proceedings, sometimes byzantine but also sometimes uplifting, can be objective, so that many other participants will have their own versions of what follows. It is, however, a fact that none of the seventy people present dissented from the proposition that there is something to discuss. The proceedings opened with statements of the need to know how to live with technology from Mr R. O. Anderson, chairman of the Aspen Institute (who is also an Atlantic-Richfield oil man and on that account especially concerned about (a) pollution and (b) natural resources) and Mr Joseph Slater, President of the Aspen Institute and also of the Salk Institute over the next row of mountains to the west. On reflexion, it was probably a mistake to begin with what was billed as a dialogue between the co-chairmen of the meeting, Dr Alexander King (OECD, Paris) and Professor M. Gell-Man (California Institute of Technology). There is a limit to the number of times on which people can say to each other in public "Well, Murray", or "Well, Alex" and still retain a serious demeanour, at least if they are not professional actors. One curious effect of this format was to create an impression of alarm among the audience which the speakers themselves say they had not intended. Is

there a crisis, and is it a crisis of the environment? Some thought not, and thought that the actors had gone beyond the bounds of reason. Others thought yes, but thought the actors had been too mealy-mouthed. What did they really mean to say? This uncertainty, neither a conviction of doom nor cheerful optimism, seems to have cast its spell over the three days that followed. In circumstances like these, it is only fair to say that some useful work was done. Closer knowledge rather than deliberate choice means that there is more to say about the group of people worrying about "problems of economic and demographic growth". The chairman, Professor Roger Revelle, the oceanographer turned demographer who is the director of the Harvard Center for Population Studies, is a mine of information about natural resources, fertility rites the world over and even carbon dioxide in the atmosphere. He ran a comparatively optimistic group of people through a short and narrow course in which numbers and principles were mixed more or less to suit all tastes. What were the results?

First, actual food supply is not a limitation for the world's population—the earth could support 50,000 million people if only existing agricultural techniques were applied most efficiently to all cultivable land. Moreover, there are already signs that the birth-rate is beginning to decline in those small developing countries where the pace of economic development is greatest. But Singapore, Hong Kong, Taiwan, Jamaica and even Albania seem to have paved the way for falling birthrates to follow elsewhere once economic development gets under way. The moral, everybody in the group agrees, is to encourage economic development not merely for its own sake but also as a means of bringing population to a stable level. People hope that if all goes well, the population of the world might settle down at 15,000 million a century or so from now.

Why bother? Is the danger the lack of resources? Quickly it turns out that fears on this score are even less serious than the danger that food will be hard to grow for a population of 15,000 million, even if there will have to be a great deal of substitution (such as aluminium for copper or uranium for oil). At least from this comfortable distance, the chief reason for hoping that populations will not grow too quickly is that there is bound to be a trade-off between the rate of growth of a population and the rate at which it can improve its economic and social lot. Neo-colonialist though this sentiment may seem in other circumstances, nobody raised a voice in protest.

But what about the danger of spectacular disasters? Carbon dioxide and the greenhouse effect is at the top of every doomsday list. By all accounts, there has recently been a solemn study of the likely accumulation of carbon dioxide in the atmosphere from the burning of fossil fuel, and it turns out that the exhaustion of the world's reserves of fossil fuel would increase the

carbon dioxide content of the atmosphere by 25 per cent and the temperature at the surface of the Earth by about 3 degrees Fahrenheit (which is, of course, enough to make climatologists lie awake at night). But then everybody agrees that what matters is the rate at which carbon dioxide is released into the atmosphere, and that a moratorium on the burning of fossil fuel would mean that the concentration would depend on the balance between the oceans (which are a sink for carbon dioxide) and the release of gas from volcanoes and the weathering of rocks, with the biosphere holding the balance in between. In short, stop burning fuel and the carbon dioxide will be what it has been for most of the past 10,000 years at least. Everybody heaves a sigh of relief.

Resources, however, are only half the problem. What about capital and foreign aid? This is where the gloom begins. Will the United States ever again be able to increase the amount of money spent on helping economic development elsewhere? Or will Western Europe have the will to take up the fallen mantle? Nobody can tell, but one African participant does vigorously affirm that there is no point in merely building an aluminium smelter alongside the Volta dam if it only uses local resources (in the form of hydroelectric power) and not even local bauxite and does not contribute to the skills of Ghana. Liberal men and women from all over the world agree that aid-giving countries must lean over backwards to allow developing nations to find their own way forward, that organizations such as the World Bank should be even more willing than at present to allow social benefit as well as financial profit to be a consideration in making loans and that it rings hollow for developed nations to urge on developing nations the attractions of international trade only to erect tariff barriers to keep out foreign goods whenever domestic industries are threatened. Developed nations, in short, should settle for more advanced forms of technology.

But what of the developed nations? These, after all, are the lands in which the SST will fly. To begin with, there is great favour for the belief that avoiding nuclear warfare is the first objective. From the group with Mr Harvey Brooks as chairman and from others, there is also great enthusiasm for the practice of what Mr Brooks himself has called technological assessment, as well as for the somewhat more nebulous "techniques of intervention in technological development and deployment by control, regulation, or the provision of positive or negative incentives in a manner sufficiently flexible so as not to inhibit or discourage beneficial innovation." In short, if the SST is a nuisance, tax it. This group, like that brooding on developing countries, is destined to feel the sharp edge of Miss Mary McCarthy's tongue—Panglossians, optimists and relativists to a man, she calls them.

Other groups have less cheerful conclusions to report. Those concerned about the ways in which technological developments could be controlled consider that affluent communities and societies make the pace—the poor and disadvantaged cannot afford such doubts. To what extent does this stem from some sense of exclusion from the decision-making process? And is there a danger that the making of decisions on technical matters will come to be divorced from the political process? People are against "creeping back-room technology" as one person calls it, although

nobody goes as far as to fix this label on Mr Brooks's technological assessment. So people went on to ask for better regulatory institutions and to assert that there is nothing wrong with parliamentary democracy that participation could not cure.

Educational institutions are a splendid case, and Dr Ivan Illich, philosopher and one-time Jesuit from Mexico, startled everybody with tales of how many of the communities in South America could afford neither the practice of classical education nor the social straitjackets which it makes. Why not, instead, some mixture of apprenticeship, skill training and motivated literacy training, for is not the classical system (as followed in the United States) an invention of the middle class? Indeed, is not even childhood such an artefact? Everybody wonders about this, hoping only that Dr Illich will find some way of making sure that people can escape from their environments if they wish.

With hindsight, no doubt, the organizers are probably convinced it was a pity not to have brought these opposing views together, but none of that explains why the conference should finally fall flat on its face in an attempt to hammer out a statement. For are not respectable differences of opinion tolerable or even enlightening? A part of the trouble seems to have been the belief that the circumstances called for a manifesto, not a summary of the proceedings. Another is the

MEN have from the beginning used technology to alter the physical and social environment. One should not underestimate that accomplishment. Technology has eliminated hunger and misery in many countries, and it can do so in many more. It has helped to halt malaria, polio, famines, and floods. It has provided unprecedented opportunities for education and for the development of individuality.

Yet technology has also served the interests of suppression, genocide, saturation bombings, and economic exploitation. In other words, technology can become the tool of mindless, selfish, or malign governments or industries that overlook human ends. Today, moreover, technologies are so powerful that they can threaten radical and irreversible changes in the entire planet, in the quality of human life, and even in the biological nature of man. The root of the problem is not in technology as such, but in its generation, its management, its use, and in the difficulty of controlling it.

We are concerned both about the sense of defeat shown by many young people and poor people and about the complacency of large sections of the public in all countries. We are also concerned about the continuing deterioration of many parts of our natural environment.

We are all agreed that the problems of poverty and human deprivation are the most pressing we now face, but we are also unanimous in the belief that there will be an environmental crisis if we do not take deliberate and timely steps to prevent it. —extracts from a statement adopted by the majority at the conference.

strange zeal with which one of the chairmen, Professor Murray Gell-Man, is forever sponsoring alternative draft documents to whatever the rest of the conference has agreed to commission. The first collision between an official draft and one tucked away in Professor Gell-Man's sleeve is the commissioning of yet a third, which is in turn capped by an armful of Xerox copies from Professor Gell-Man, rich with mindless phrases such as "mindless, selfish or malign governments". In the end, those not driven into the sun by the rephrasing of the sentences find themselves agreeing that the statement as finally issued should even make obeisance to the women's liberation, but in the small hours and the umpteenth redrafting, this laudable sentiment is left out. Is there something about typewriters that can spot irrelevancy when people are exhausted? Will there ever again be such a splendid chance for finding out?

TERRORISM

Bombing at Madison

from a Correspondent

ON August 24, just three weeks before the fall academic session was about to begin at the University of Wisconsin, Madison, terrorists blew up the Mathematics Research Center. One graduate student was killed, four persons (including a graduate student from South Africa) were injured and \$6,000,000 in damage was caused. The bombing capped a 19-month period in which the National Guard had been called up three times—twice for campus disruptions and once to guard the nearby capital of the state of Wisconsin. Madison, a city of 170,000, once was called by *Life* magazine the best place in America to live and study. The then picturesque, tranquil campus of one of the nation's leading public universities was cited as an asset in that article more than two decades ago. Tension is high and optimism is low in Madison these days. There are doubts whether the university will be able to complete its fall semester without serious incident. Students are reported to be stockpiling weapons; and a rather large "hippie colony" has made the city its home, much to the concern of the city's establishment.

The bombing took place at 3.40 am. At about midnight, a nuclear physicist, Robert Fassnacht, 33 years old, had gone to work to finish up some research before a scheduled vacation with his wife and three children. His body was found beneath the rubble. Data books of five postdoctoral colleagues were also buried, and their loss has in some cases set back the students by as much as three years. The physical sciences community across the nation was shocked by Fassnacht's death. Until now, by and large, social scientists and humanists have been those mainly involved in, and affected by, the student revolt. It is the scientists who have now been provided with dramatic evidence that they are not immune from the capricious terrorism of youths bent on destruction of research centres.

Later, a militant radical group called the New Year's Gang spread the word, through an underground newspaper story, that they did the bombing. Four young men, three of them school drop-outs and the fourth a former editor of the student newspapers have been charged with the bombing by the FBI.

Demonstrators on campus streets insisted that the

bombing was done only after a year of other protests had been futile; that the research centre did work directly on projects related to the Indo-China war, that the bombing had been a humane act—designed to save lives of Vietnamese, Cambodians, Laotians and American soldiers. Ironically, many of the researchers who worked in the demolished Sterling Hall also were "doves" but they did not believe in terrorism. The New Year's Gang has threatened that, if its demands are not met by October 30, 1970, there will be kidnapping of public officials in the Latin American style. Its demands include abolition of the Army Reserve Officer Training Corps centre on the Madison campus as well as the "elimination of the male supremacist women's hours at the university."

Sterling Hall was and is the University of Wisconsin's main physics building. The basement and first floor house the school's physics experiments and these were most heavily damaged. As part of the physics department, there is a 10 MeV accelerator financed by the US Atomic Energy Commission. The second, third and fourth floors are used by the Army Math Research Center though the third floor is shared by the university's multi-million dollar computer centre. Floors five and six house the university's astronomy department.

The biological sciences also were affected by the bombing. The School of Pharmacy building is located across the street from Sterling Hall. Dean David Perlman estimated that about \$1,000,000 in damage had been done to the pharmacy building. "One cannot equate in dollars and cents the loss suffered in the areas of cancer, Parkinson's disease, antibiotics, and many other facets of pharmaceutical research lost due to the bombing," he said.



Sterling Hall Physics Building.

The Mathematics Research Center was established about 15 years ago with financial support provided by the United States Army. Its mission was to do basic research in applied mathematics. Wisconsin was one of several universities that had placed a bid to have the centre located on its campus. The centre has been host to mathematicians from many countries of the world, including Yugoslavia and Egypt. Under the centre's arrangement with the US Army, support is given to foreign scientists who could not otherwise obtain funds from even such US research agencies as the National Science Foundation. All of the work at the centre is

unclassified, and available to all who ask or pay the purchase price of books or research papers in which results are published. Professor J. Barkley Rosser, director of the centre, says that "none of the work is in any sense secret. The problems on which we work are basic and usually pertinent to many fields of science".

For several years, the Army Math Center at Madison has been the target of picketing and sit-ins by young radicals, some of them students at the university, others just gypsy-like dissenters who wander across the nation looking for causes, usually identified as anti-war crusades.

On February 12, 1970, students broke windows in the Math Center during demonstrations against the General Electric Co., which had sought personnel recruits on the campus. The student dissidents accused GE of being a major defence contractor. Windows were broken in the centre a week later as a protest at the outcome of the Chicago 7 conspiracy trial. Last April 18, when President Nixon ordered the invasion of Cambodia, the building was attacked again. Thus, within a month, a building that had relative peace for nearly 12 years—it was built three years after the Math Center was established—became a target for student dissent. Rosser, who likens the centre to a "think tank", says that the centre received more than \$1,000,000 in support from the Army last year.

Aware of the negative image of the Math Center on the campus, a 32-page detailed explanation of its work was sent to all members of the Wisconsin faculty last fall. The booklet described activities such as the centre's mathematical studies of what happens when a hole is punched in a sheet of metal or plastic. The centre has also undertaken studies of the properties of elastic substances, and the mathematics of wave action.

COMPUTERS

Living with Machines

from our New York Correspondent

A COMPUTER conference "committed to the proper management of computer technology for the greater good of mankind" was held last week in New York City. The 25th national conference of the Association for Computer Machinery stressed that scientists care about society and attempted to create dialogues between computer experts and computer users. At times the dialogue dissolved into competing monologues, but the will was there, and hopefully the way will follow.

The first day of the conference considered the application of computers to urban problems. Marvin L. Mannheim, a civil engineer at the Massachusetts Institute of Technology, delineated what he called the "myth of rationality", the belief that there is a single best course of action and that the computer can determine it. There can be no such thing, he said, since any definition of what is best is determined by values that differ for every group. Value discussions are hidden in everything we do, Dr Mannheim added, and since it is far more difficult to define the qualitative values than the quantitative, the latter are usually emphasized though the former are probably more important. Even when a programme attempts to assign numbers to social and aesthetic functions, it proves to be an almost impossible task.

David Grossman, the Deputy Director of the Budget for the City of New York, argued that the political process is already closely allied to decision-making but often there is too little guidance of any sort, so that what is really needed are measurements that could be looked at with a cold eye. Dr Mannheim agreed that this was one of the potential benefits of computer use in this field and narrowed his attack to those limited studies that only focus on parts of a problem in the belief that a computer can provide the total information necessary for reaching a decision. He felt that all a computer could attempt to do was fill in the blanks in the statement, "these are the key courses of action that could be taken, with these interest groups gaining and these losing."

Urban hardware was the theme of John P. Eberhard, Dean of the School of Architecture and Environmental Design at Buffalo. "The second generation of urban hardware now around us has replaced the first generation that was in operation for thousands of years," he said, "but all of the necessary components—steel, elevators, central heating, indoor plumbing, electricity, the automobile, and the telephone—were developed between 1880 and 1892." We are now in the process of a shift from this second generation to a third, and the old generation must eventually die. The computer industry must be prepared for the challenge of this third generation and must address itself to the problem of being a part of it.

An address by Ralph Nader, the consumer advocate, broadened this appeal to include all problems outside the realm of normal market forces, and brought him a standing ovation from the audience. He called for an information "Bill of Rights" to ensure that the efficiency of the computer's ability to gather and store information does not outpace the development of legal controls preserving society's right to privacy. He warned that if this does not come about soon there will be mass antipathy to the whole technology; "never underestimate the power of the citizen backlash to something it doesn't understand and feels is taking unfair advantage."

The legal question regarding privacy will soon become a major issue, Mr Nader predicted, because it must determine to what extent it is necessary to maintain barriers around people's lives in order to keep society functioning. Legal issues also arise in the area of monopoly power in the computer industry, for there is a danger that when new technology is dumped into an existing corporate structure, an archaic corporation may well block its own use of new advances and at the same time stop new companies from using these advances in dynamic ways. As computers are given increasingly complex tasks in this very interdependent society, they must be able to correct themselves and try new approaches if the entire system is not to be "gummed up", he said. "With such a complex technology, the professional societies must be the first to determine if adequate safeguards are being taken". Mr Nader also noted that "We are entering a period where we can almost program innovation, but the question is, are we going to apply this innovation to those areas that most need it?". Professional standards must provide criteria for choosing between professional, corporate, and personal disputes. "When a professional society allows its standards to atrophy, is the atrophy of the profession itself far behind?"

NEWS AND VIEWS

Crystallographers' Progress

Now that the first shining successes of protein crystallography, lauded in song and story, have passed into history, the applause that greets the unveiling of each new model of a hydrolytic enzyme is becoming a trifle perfunctory. To stimulate the jaded intellectual palate something more overtly spectacular is evidently needed, and on p. 1098 of this issue of *Nature* we are happy to present just such a confection. Rossmann and his team have achieved a 2.8 Å map of no less an enzyme than lactate dehydrogenase, which has a molecular weight of 140,000, with more than three hundred residues in each of the four subunits. The reader, contemplating the stereograms with sagging jaw, might wish to be reminded that NAD is a cofactor for this enzyme, and that it binds independently to one site on each subunit. The high-resolution structure that Adams *et al.* now present is that of the apoenzyme.

The subunits have a compact form, with a cleft down the middle, containing the binding site for the adenylate function of the NAD, which, as recent work by McPherson from the same laboratory has shown, acts as a conformational trigger: its attachment engenders a structural transition to a state with high affinity for the nicotinamide end of the cofactor. The dodecapeptide that has been snipped out of the protein and contains the enzymically active thiol group has been identified, and lines one wall of the cleft. About a quarter of the residues are distributed between eight α -helical segments, and for connoisseurs of polypeptide structure there are some fragments of the rare and desirable 310-helix; 10–15 per cent of the residues are also in the β -form, some in the antiparallel, and some in the parallel form, which has never been found in isolation (as in fibrous proteins), but has been observed in the subtilisin molecule by Kraut and his co-workers. Most of the recognizable secondary structure occurs in the N-terminal half of the chain. Side-chains which are in a position to make contact with the cofactor have been tentatively identified, and Adams *et al.* note that some residues known to be functionally important in another dehydrogenase enzyme, glyceraldehyde 3'-phosphate dehydrogenase, occupy similar positions in the sequence to some that cluster round the cleft in the lactate dehydrogenase subunits. On this basis one might hazard the surmise that the stereochemistry of the active site could well be similar in the two species.

The real pay-off from this work, of course, lies in the future, when the holoenzyme and its inhibitor complexes are also solved at atomic resolution, so as to allow a detailed comparison. From the available data at 5 Å, the general form of the conformational convolution that accompanies the binding of the cofactor has already been defined (Adams *et al.*, *J. Mol. Biol.*, **51**, 31; 1970). In their new report in this issue, the authors

also describe the important step of crystallizing an abortive complex of the holoenzyme with the substrate, pyruvate. From the results at 5 Å, it is already clear that the introduction of the latter brings about a considerable displacement in one part of the molecule—a loop of twenty-four residues making up the mouth of the cleft. This migrates 12 Å, thereby closing the cleft so as to occlude the nicotinamide ring. A change in electron density also occurs in the vicinity of asn-150 and ser-147, which form part of the fragment containing the essential thiol group. Enzymologists will wish Rossmann and his colleagues a swift ascent to the next daunting peak.

PROTEINS

Independence and Togetherness

from our Molecular Biology Correspondent

THE problem of cooperative binding phenomena in proteins, which not so long ago seemed on the verge of illumination in terms of a number of simple generalities, has now, alas, receded once more into a malodorous miasma of complex and contradictory observations. Even the archetype, haemoglobin, which has probably claimed more man-years of research than any other protein, has not been spared. A fresh attempt to impose a simple formalism on the kinetics and equilibrium of the oxygenation reaction has now been made by Gibson (*J. Biol. Chem.*, **245**, 3285; 1970). He has expressed the uptake of oxygen by the four haems in terms of four independent consecutive steps. The data, in other words, must be accommodated by eight rate-constants, four each for the forward and reverse reactions. Now in terms of the varyingly attributed quip that with four independent parameters one can draw an elephant, this seems at first sight a dispensable exercise. But, on closer inspection, a number of restraints reveal themselves. In the first place, the rate constants for the reaction of the fourth oxygen can be independently determined from measurements of the kinetics of replacement of oxygen by carbon monoxide, the carbon monoxide system being kinetically defined by the early work of Roughton and Gibson. The remaining six rate constants must then be selected so as to satisfy a family of curves for the binding and dissociation of oxygen at different levels of saturation. The ratios of the on and off-rate constants must, moreover, be such as to yield binding constants which will fit the equilibrium curve.

These requirements between them are sufficiently restrictive that a set of what one may hope are unique rate constants can be computed. Gibson concedes that he has defined the system only in operational terms, and that more states than the five which are implicit in this scheme must be presumed to exist. It is, however, seen that the cooperative binding of oxygen can be accounted for by the different values of the rate constants. Moreover, their relative magni-

tudes, which imply, for example, a large affinity change after loss of one oxygen, are not readily compatible with a two-state, or allosteric, model which, as this and other evidence indicates, is unlikely to apply to haemoglobin. Moreover, the kinetic basis of the important effect of phosphates, in particular, 2,3-diphosphoglycerate, on the oxygen uptake curve has been determined: the velocity of dissociation of the first oxygen is unaffected, but the other three rate constants for dissociation are greatly diminished when the cofactor is excluded.

A strange case of cooperativity, albeit of a rather low order, occurs in the haemoglobin of the lamprey, which in the liganded form in dilute solution is a monomer, but associates when deoxygenated. Rumen and Chance (*Biochim. Biophys. Acta*, **207**, 404; 1970) have now examined the reaction of this protein with carbon monoxide in the crystal, where it is in the associated state both in the presence and absence of ligands. The experiment is possible because, unlike mammalian haemoglobins, the lamprey pigment has the same crystal symmetry in all states, so that the crystals do not break up on withdrawal of the ligand. An indubitable cooperativity is observed, and Rumen and Chance infer that the haem-haem interactions are not in this case mediated by a relative displacement of subunits within the tetramer as in horse haemoglobin, for example, for otherwise, they reason, the crystal habit would be expected to change. Neither could one envisage subunit exchange processes in the crystal. Just what degree of structural change a lattice can tolerate is a matter of speculation, however, but it is altogether possible that a quite different mechanism of haem-haem interaction might operate in the crystal from that in solution in lamprey or mammalian haemoglobins.

An extraordinary enzyme, in which the cooperativity of ligand binding can be controlled by the presence or absence of potassium ions, is aspartate-homoserine dehydrogenase of *E. coli*, which has been studied by G. N. Cohen and his collaborators. Several conformational manifestations of ligand binding have been recognized, and now Heck and Truffa-Bachi (*Biochemistry*, **9**, 2776; 1970) have made some interesting observations, using circular dichroism as a conformational indicator. Binding experiments with aspartate and the inhibitor, threonine, show that when potassium is absent and there is no cooperative effect, there are only trifling changes in circular dichroism, which are confined to the aromatic region of the spectrum. With cooperative binding in the presence of potassium, on the other hand, some quite sizable conformational effects are observed, the ellipticity changing sigmoidally with ligand concentration. The spectra can be used to define the high and low-affinity states of the enzyme (which seems to conform with a two-state scheme), and the circumstances under which either one predominates are nicely reconciled with earlier inferences. The Cotton effect associated with the cofactor, NADPH, also responds to the attachment of ligands.

DNA SEQUENCES

Slipped Circles and Sticky Ends

from a Correspondent

ONE of the continuing triumphs of present day molecular biology is the increasing understanding of the fine

structure of the genome of eukaryotic cells. The cytological studies of lampbrush chromosomes by Callan were a major starting point, and led him to suggest that in many instances numerous copies of each gene were present in eukaryotic genomes, represented as tandemly repeated sequences within the DNA. The presence of substantial amounts of sequence reiteration within eukaryotic DNA was supported by the well known studies of its renaturation kinetics, carried out by Britten and his colleagues, and it is also now clear that in various satellite and ribosomal DNAs there is tandem repetition of similar or identical sequences.

Now, in some brilliantly conceived experiments, C. A. Thomas *et al.* (*J. Mol. Biol.*, **51**, 621; 1970) have been able to demonstrate in a fairly direct way that such tandemly repeated sequences must in fact make up a large proportion of the genome of several eukaryotic species which they have studied. The experimental rationale was as follows. If a considerable proportion of eukaryotic DNA is indeed made up of identical tandemly repeated sequences, then it should be possible to convert large fragments of such DNA into circles, by limited treatment with strand-specific exonucleases to expose single-stranded regions at the ends of the fragments. These could then anneal together because parts of their sequences would be complementary. Thomas and his colleagues term this procedure "folding". An alternative way of producing circles would be to denature the DNA fragments and then re-anneal them. In repetitive DNA, "slippage" between the strands would be likely to occur so that single-stranded ends would be produced which could form duplexes with each other. In both instances, it would be possible to see the circles by electron microscopy.

They put theory into practice with DNA from *Necturus*, trout sperm, salmon sperm and calf thymus. Degradation of sheared DNA with exonuclease III (exposing 5'-ended single strands) or λ exonuclease (exposing 3'-ended strands) to a varying extent (5-25 per cent) followed by annealing yielded a plethora of circles in all cases, with some lariots and polyrings to boot. The overall frequency of circular DNA structures was not less than 20 per cent and as much as 35 per cent in the case of *Necturus*. Numerous examples of the structures are shown in some extremely attractive electron micrographs. The necessary control experiments which were carried out with undigested fragments and with prokaryotic DNA (from *Escherichia coli*, *Bacillus subtilis* and bacteriophage T7), gave far lower values (around 5 per cent for the undigested controls and 0.5-1.4 per cent for the prokaryotic DNAs). Similar results were obtained with the slipped circle technique, although no quantitative studies could be carried out because of the large fraction of DNA remaining denatured.

Thomas *et al.* also studied the interesting question of whether these repeated stretches were distributed throughout the eukaryotic genome, or whether they were particularly associated with any compositional class of the DNA. They found, at least for *Necturus* DNA, that all the fractions which they took from a peak on a CsCl gradient possessed a similar ability to cyclize. The dimensions of the circles could be calculated from the electron micrographs. Most of the circles were less than 2 μ m, but some were 5 μ m or even 20 μ m in circumference. They hazard a guess that some of the gene families in trout DNA encompass 4-5 μ m, and as much as 8-10 μ m in *Necturus*. Thomas

et al. suggest that the efficiency of the cyclization is only 50 per cent on a generous estimate. Therefore, as much as 40–70 per cent of all the fragments of DNA contain tandemly repetitious sequences. How such sequences are kept identical remains a mystery.

Meanwhile down at the bacteriophage genome (still no mean problem), R. Wu reports (*J. Mol. Biol.*, **51**, 501; 1970) his *tour de force* with the nucleotide sequences of the sticky ends of the DNAs from bacteriophages λ and 186. His method, which is potentially a general technique for the nucleotide sequence analysis of DNA, was to build the complementary strand on to the single-stranded regions which protrude from the ends of the DNA duplexes, and are a common feature of bacteriophage DNAs. He used *E. coli* DNA polymerase and ^{32}P -labelled deoxyribonucleoside triphosphates to synthesize these complementary strands *in vitro*. In the first instance he determined that the cohesive ends in the λ and 186 DNA each contained twelve and seventeen nucleotides respectively, from measurements of the incorporation of the radioactive nucleotides. He was able to deduce some of the nucleotide sequence of both the λ and 186 ends by nearest neighbour analysis, using only one out of the four radioactive triphosphates in the incorporation. Some further help was obtained from the fact that the two cohesive ends are known to be entirely complementary to each other in both bacteriophages. The *coup de grâce* (well, nearly) was achieved by digesting the DNAs with micrococcal nuclease, and partially digesting the various labelled terminal fragments so obtained with venom phosphodiesterase. He reports two thirds of the sequence of the λ DNA ends, pGGCGGCG being the sequence complementary to the inner eight nucleotides in the right-hand protruding strand, pCGCCGCC. In 186 DNA, the first four nucleotides added to the right-hand end had the sequence pGGCG.

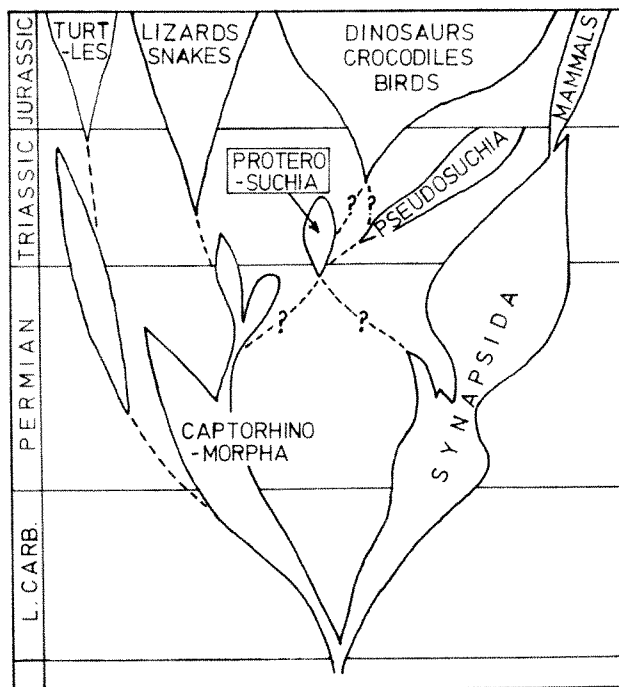
ARCHOSAURS

Divergent Evolution

from our Vertebrate Palaeontology Correspondent

THE course of evolution of mammals from synapsid (mammal-like) reptiles is now known in enough detail that a number of papers have been devoted to the problem of where to draw the taxonomic dividing line between the two groups. The ancestry of the great archosaurian group of reptiles, which includes dinosaurs, crocodiles and the ancestors of the birds, is far more uncertain. This is partly, at least, because the earliest known archosaur group, the Lower Triassic Proterosuchia, comprises two already divergently specialized types. Furthermore, even the composition of the Proterosuchia, as well as the classification of the proterosuchians themselves, has been very differently viewed by past authors. These facts add complexity to the problem of defining the ancestry of the archosaurs as a whole, and also to the problem of the precise evolutionary pathway between the early archosaurs and the more advanced, later forms.

A welcome first step towards solving these problems is the recent discussion of the composition and classification of the Proterosuchia by A. J. Charig and O. A. Reig (*Biol. J. Linn. Soc.*, **2**, 77; 1970). The two different types of proterosuchian are exemplified by *Chasmosaurus* and *Erythrosuchus*. The former is a medium



Ancestry of the Archosauria.

to large sized, slender animal, crocodile-like in appearance and probably also in its way of life, with a long, low skull, a long neck and a short tail. *Erythrosuchus*, on the other hand, is a large to very large, clumsily built carnivore, rather like a hippopotamus in its general build; it has a massive skull, a short neck and a long tail. The fact that *Chasmosaurus* is found slightly earlier than the first known erythrosuchid, and the existence of two genera which bridge the evolutionary gap between the two families, suggests that the erythrosuchids may have evolved from *Chasmosaurus*-like forms.

In another, more controversial paper, Reig tackles the problems of the ancestry of the proterosuchians and of their relationship to the pseudosuchians and saurischian dinosaurs (*Bull. Mus. Comp. Zool. Harv.*, **139**, 229; 1970). It is commonly believed that the archosaurs evolved from the earlier captorhinomorph reptiles, which are primitive in many ways. Though he agrees that this is possible, Reig believes that a better case can be made for the evolution of the proterosuchians from the varanopsids—members of the synapsid group of reptiles. He points out that the varanopsids possess a number of characteristics found in the proterosuchian archosaurs, such as the position of the jaw articulation, well posterior to the level of the occipital condyle, so that the occiput slopes forwards and there is no notch for the tympanic membrane, and also the presence of an ant-orbital opening in the skull and possibly also of an opening in the side of the lower jaw.

Palaeontologists are likely to view Reig's theory with considerable scepticism, because it would imply that the peculiar spiral division of the base of the aortic arches (which is found in all living reptiles) must have evolved twice—once in the captorhinomorph ancestors of turtles, lizards and snakes, and again in the line leading from the synapsids to the archosaurs (including crocodiles and birds). This would

follow from the fact that this specialization of the aortic arches is not found in the mammals nor, by inference, in their synapsid ancestors. Unfortunately, Reig does not even mention this difficulty. He also believes that the proterosuchians are the direct ancestors of both the crocodiles and the sauropod dinosaurs, whereas most other workers believe that these groups evolved from the Pseudosuchia. Whether right or wrong, Reig's views will stimulate further discussion and argument.

COMPUTERS

Human Needs are What Matter

A NEW way of teaching children how to think with a computer was described by Professor Seymour Papert of the Massachusetts Institute of Technology at a conference on man-computer interaction held at Teddington, London, from September 2 to 4. His idea is that by letting children re-invent the rules of geometry or grammar through a computer program they will become instilled with the same sense of excitement experienced by scientists and technologists.

One of the evils of the present educational system, according to Professor Papert, is that it classifies children as failures if they cannot master quickly a desired subject and this causes despair and disenchantment. In his system children are taught to write their own computer programs in a very simple computer language called LOGO, and are made aware from the outset of the art of debugging a program. Professor Papert cited a simple program to design a man from a choice of geometrical operations on a set of matchsticks: by displaying the drawing on a cathode ray screen the child may retrace his steps and discover, if need be, where his geometry went wrong. Although the novelty of the scheme may have induced special interest, Professor Papert is greatly encouraged with the results of his first class of eight year olds and next year he hopes to maintain his first year-long course with a class of forty children.

However much medical practitioners may flinch at the thought, computers seem to be endowed with a charming bedside manner. This was the conclusion presented by Dr C. H. Evans of the National Physical Laboratory and Professor W. I. Card of the University of Glasgow who have been investigating how patients with suspected ulcers react to question and answer sessions with a computer. Once familiar with the button pushing routine, patients seemed to warm to the chatty program devised by Dr Evans to get patients to divulge their symptoms. Dr Evans and his colleagues are still working on how factors like response time could be related to the intuitive side of a doctor's interrogation, but Dr Evans pointed out that there seem to be some important advantages in using computers, apart from saving hospital time. A difference in class between doctor and patient can seriously inhibit communication, he said, and doctors are sometimes also irritable and tired. Computers are cool and classless, on the other hand.

It may soon be possible to translate mechanical shorthand into normal English on a computer, according to Dr W. L. Price of the National Physical Laboratory, which would provide barristers with on-the-spot transcripts of evidence and speed up the production of

official documents such as Hansard. Several major hurdles still have to be overcome to transpose the twenty-nine symbols of palantype, the British form of mechanical shorthand, into proper English. The computer operates by comparing the incoming sounds with a dictionary of words, and difficulties arise with homophonic words like "insight" and "incite". The group at NPL have programmed in a limited amount of syntax analysis by allowing for the scanning of adjacent words when an ambiguous word appears, and although the system is still too imperfect for satisfactory usage, Dr Price is hopeful that a viable version can be produced.

Work is also in progress at NPL on speech recognition by computers, a yet more hazardous task. Professor C. Longuet-Higgins of the Machine Intelligence Unit at the University of Edinburgh maintained indeed that the chasm between the inconsistencies of natural language and the cold logic of computers was so wide that it was wiser not to try to bridge the gap. "Computers are ideal tools for studying natural language," he said, "but not for understanding it." He stressed that comprehension of English rests on an immense fund of common knowledge between conversants, and he mocked the computer's innocence in the face of the two statements, "Nothing is better than a good square meal", and "A sandwich is better than nothing".

SEISMOLOGY

Numerical Earth

from our Geophysics Correspondent

THE International Symposia on Geophysical Theory and Computers have, since their inception, been fairly small affairs, rarely attracting more than a hundred geophysicists, unlike the preposterous jamborees that most international meetings have become. The reason for the relative smallness is the highly specialized subject that is being dealt with, but out of this modesty in size comes also a quite remarkable camaraderie. The atmosphere is such that it is really true that coffee table conversations get converted into research effort and next year's papers.

This year's meeting, the seventh in the series, was held in Stockholm from August 17 to 28. Most aspects of geophysics were covered, but as usual seismology was strongly dominant. Problems of data inversion, wave propagation and array processing received most attention. A notable exception was a paper by T. Dahlen (Cambridge) on excitation of the Chandler wobble by earthquakes. This controversial topic draws on the whole range of applied mathematics, before anything meaningful can be said. Dahlen dealt with a "realistic" Earth model and showed that the changes in products of inertia arising from displacements caused by large earthquakes are compatible with the amplitudes of renewal of Eulerian nutation (a few metres shift in the North Pole) frequently observed. Dahlen remarked, however, that there are still difficulties of analysis and interpretation which need more refined study of the data on which these ideas are founded.

Another paper which attracted much attention was by R. Burridge (Cambridge) on the form of radiation from earthquake faults. This problem is at best

difficult to handle theoretically, let alone numerically, so the work presented was of great interest in that it now seems possible to specify plausible fault models and predict signal waveforms. The interaction of a fault with the free surface was shown to produce the most striking features in the far field radiation.

Techniques for inverting geophysical data and for making statements about the uniqueness or resolving power of such inversions have received wide study in the past five years following the complementary attacks on the problem by Backus and Gilbert in La Jolla and Keilis-Borok's school in Moscow. Papers on inversion and a panel discussion were presented by T. Jordan and D. L. Anderson (Caltech), L. Knopoff (UCLA), F. Press (MIT) and V. Keilis-Borok (Institute of Physics of the Earth). The subject is appealing in that a broad range of attacks can be mounted on it, from the austere theoretical study to the Monte Carlo method. In addition to constraints imposed by the actual data, there are also the somewhat more difficult to inject constraints of relationships between elastic parameters and densities, and "smoothness" criteria. These are matters of much discussion, and the whole led to a thoroughly stimulating session which undoubtedly will be repeated in future years.

A subject which has seen rapid growth in the past few years is array processing of the vast streams of seismic data which are now recorded especially from the Large Aperture Seismic Array (LASA) in Montana and NORSAR in Norway. Papers by R. Baron and S. Francisco (IBM, Washington) described the extensive processing necessary for routine acceptance of such data and H. Bungum (Norwegian Defence Research Establishment) outlined the NORSAR installation. D. Davies (MIT, Lincoln) gave a description of some research uses of arrays in the study of inhomogeneity in the mantle. It has been suggested that arrays, like many other technological innovations, are a tool in search of a user. Extended discussions were held to allow a panel of research workers connected with arrays—E. Husebye (Norway), E. Flinn (Geotech Corporation), D. Corbishley (UK Atomic Energy Establishment) and Davies to put forward and receive new ideas on array applications.

Of the many other papers presented, perhaps the most interesting was a didactic presentation by J. Bazer (Courant Institute, New York) on magneto-elastic wave propagation in both linear and non-linear cases. This field is arguably the most difficult to comprehend of all wave propagation cases and it was greatly to the credit of Dr Bazer that the subject was put over so lucidly to his audience.

In the past the symposium has lived under the financial umbrella of the Upper Mantle Committee, but with the imminent demise of this committee future success will depend heavily on the capability of raising \$15,000 every other year. The scientific merit is assured through the remarkable talents of the organizing secretary, Dr Keilis-Borok, but an imaginative sponsor would be another great asset. This year, through the singular efforts of Dr Bo Jansson, industrial support was forthcoming, particularly from Svenska Handelsbanken and IBM (Sweden). The plans are to return to Stockholm for future meetings and to try to encourage graduate student attendance by travel grants. Potential sponsors who profit by the number-crunching of this group and would like to add their name to the title of this meeting, please note.

WATER CONSERVATION

Natural Reservoirs

from our Geomagnetism Correspondent

THE obvious way to store water for future use is to build a dam. That is man's way; but nature has its own, more impressive, techniques. Ninety-six per cent of all freshwater on the continents is stored below the ground surface in aquifers. To be sure, some of the water which falls onto the continents is lost in run-off; but in the United States, for example, the 7,300 cubic miles of water which rivers annually discharge into the sea represents only 0.4 per cent of the total stored in sub-surface aquifers.

But can man make use of nature's store? The answer to this question is that not only is it possible but in the United States and Israel it is being done. According to Dr Charles L. McGuinness of the US Geological Survey, aquifers now supply about 61,000 million gallons a day, about a fifth of all water used in the United States. Indeed, in some areas, notably southern California and Long Island, New York, more water is being removed from aquifers than nature is replacing. Accordingly, it has become necessary to replenish ground water supplies artificially.

Artificial recharge is being achieved in a variety of ways including injection into pits and wells and the use of structures which divert and spread concentrated water supplies such as rivers, thereby increasing the area over which water can percolate downwards. The precise technique adopted in any given case depends on the nature of the water sources, the sub-surface geological structure, the pattern of subsequent water usage and, of course, cost. Clearly, the sub-surface rocks must not be impervious to water, but at the same time they must not be so porous that the water moves around too freely. And as far as cost is concerned, simple spreading structures are preferable. They are used in California, for example, to give the water more time to filter into the alluvium at the mouths of canyons. In the mid-west and east, on the other hand, conditions are not favourable for water spreading, and relatively expensive injection into wells must be adopted.

Underground reservoirs have several advantages over dams. There are no construction costs and little maintenance is required; the water they yield is relatively stable in temperature and quality; they do not take up valuable surface space which could be used for other purposes; they do not silt up; and the evaporation loss is negligible. Artificial recharge of these reservoirs has its benefits, but also its drawbacks. The obvious benefit is the saving of water which would otherwise be lost to the sea. On the other hand, injection through wells, in particular, is expensive. Another problem, recently highlighted by another US Geological Survey hydrologist, Dr Raymond L. Nace, centres on the quality of water available for injection. Water from rivers often contains sediment which rapidly clogs up wells, necessitating the additional expense of frequent well cleaning. More dangerous, of course, are the man-made impurities—not only those in the original water sources but the industrial wastes separately disposed of underground. Until the precise patterns of sub-surface water movement are known, if ever, the threat of contamination is obvious.

What neither Dr McGuinness nor Dr Nace mentions,

however, is the danger of injection at all. Presumably little harm is likely to result from pure replacement of water; but there is proven danger in injecting fluids where there were none before, however attractive or suitable the geological structure. The series of earthquakes which some years ago struck Denver as a result of underground waste disposal are still fresh in many minds. But even dams are not free from that problem as experience in India, France and Rhodesia has shown.

TECTONICS

A Caribbean Plate?

from our Geomagnetism Correspondent

ALTHOUGH the principal features of plate tectonics are now widely accepted, many details remain to be settled. This week, the US Coast and Geodetic Survey ship *Discoverer*, with its electronic sonar equipment, is being used to probe the bottom and sub-bottom of the Caribbean around Barbados to trace the extension of structural features found during previous studies and, hopefully, to gather evidence which will finally settle the question of whether the Caribbean is a separate crustal plate or is an extension of the South American continental landmass.

If the Caribbean forms a separate crustal plate, as some scientists apparently believe, there would have to be large faults along its northern and southern boundaries. The southern fault, for example, would presumably lie between the southern end of the Lesser Antilles and Venezuela. The *Discoverer*, however, made eighteen crossings over the supposed fault trace during 1968 and 1969 and found no evidence that such a fault actually exists. The data obtained so far thus support the conclusion that the Antilles double island arc system is an offshore extension of

South America. The present traverses should confirm this interpretation.

MICROELECTRONICS

High Hopes for New Device

by our Solid State Physics Correspondents

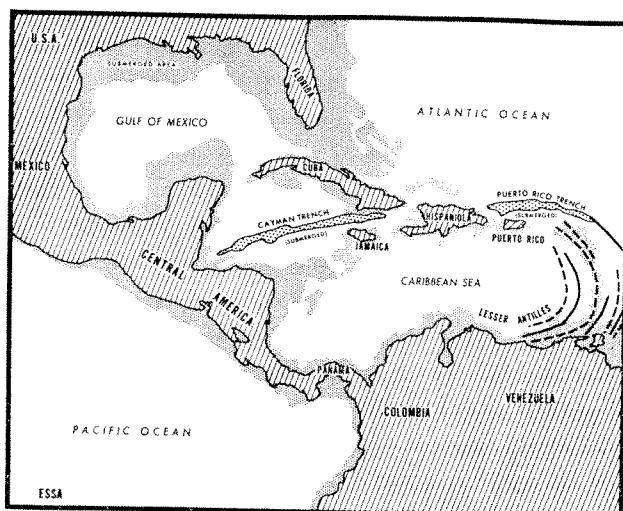
A NEW type of semiconductor device has been constructed at Bell Telephone Laboratories which promises to be of major importance in the design of computers and communications systems. It is based on an idea revealed earlier this year called charge coupling which enables a series of metal-oxide-semiconductor (MOS) elements to be linked together in a way that removes for the first time the need for separate capacitors between elements.

It is this use of the MOS element as its own capacitor for storing charge that is particularly important, both from the point of view of performance and of cost. M. F. Tompsett, G. F. Amelio and G. E. Smith (*Appl. Phys. Lett.*, 17, 111; 1970) have fabricated an eight bit shift register based on this concept, and have achieved transfer efficiencies of greater than 98 per cent for times of less than 100 ns, comparable with those for existing shift registers. They accomplished this with a design which, they say, was chosen for ease of construction rather than high performance.

The special feature of charge coupled devices lies in the way that charges are stored and passed along the device. The structure used by Tompsett *et al.* consisted of a row of MOS capacitors on a substrate of *n*-type silicon with a *p-n* junction at either end. Application of a potential to one of the capacitors creates a region depleted in electrons under the capacitor, and by varying the potential along the array the holes thus formed can be transferred to neighbouring capacitors. The key to the efficiency of this process is the generation and maintenance of a depletion region under the electrodes.

The construction of these devices hinges on the production of highly perfect oxide surfaces and silicon substrates. Although the team at Bell Telephone Laboratories seem to have overcome the major hurdle the exact way in which the performance of the array depends on different parameters in the system is still not clear. Tompsett *et al.* have found, however, that the transfer efficiency depends quite sensitively on the depth and shape of the voltage drops of the transfer pulses, and that greater efficiency is induced if the surface potential of the silicon is lowered during charge transfer.

The first experiments have demonstrated the use of the eight bit shift register as a 48 μ s delay line and as a display for an image optically focused on to the device. The most exciting possibility for these devices lies naturally in their use as shift registers and for logic operations in computers. Shift registers, after all, perform the meat of a computer calculation and are involved in the process of time sharing. Storage periods of only a few microseconds are adequate here. But the successful demonstration of the line-imaging capability of the device, in which the spatial form of an image is translated into a train of electrical pulses, must raise hopes for the optical possibilities of the system, and elevate once again the question of when a solid state replacement for the cathode ray tube will eventually be produced.



This week's investigations by the US Coast and Geodetic Survey ship *Discoverer* will centre around the Antilles double island arc system, an island arc comprising both an inner volcanic belt and an outer sedimentary belt. Part of this double island arc system rises above the water and forms the islands of Guadeloupe, Dominica, Martinique, St Lucia, St Vincent, Barbados, Grenada and Tobago. Barbados and Tobago are part of the outer sedimentary belt; the other islands are part of the inner volcanic belt. The islands, or elevated portions of the belts, are shown as broken lines; the submerged portions as solid lines.

Ion Cyclotron Resonance Mass Spectrometry

by

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This relatively new form of mass spectrometry is proving to be valuable in several branches of chemistry. This review shows that the technique has already yielded some useful data.

In a mass spectrometer, ions produced from neutral species, usually by transfer of energy from an electron beam, are separated according to their mass-to-charge ratio (m/e) and their relative abundances determined. In a standard magnetic deflexion instrument, ions are subjected to a large accelerating potential after which they are separated by passing them through a magnetic field so that only ions of a given m/e are brought to focus at the collector slit. Mass spectra are obtained by scanning either the accelerating potential or the magnetic field. Recently, increasing use has been made of ion cyclotron resonance (ICR) mass spectrometers, the mode of operation and applications of which are very different from those of a conventional analytical instrument¹⁻⁴. (The most widely used instrument is the Varian Associates V-5900 ICR mass spectrometer designed by Dr P. M. Llewellyn.) The principal area of application has been in the study of collision processes of ions which, in addition to their intrinsic interest, are important in radiation chemistry and in the chemistry of plasmas, flames and the upper atmosphere.

Principles of Operation

An ion which is moving in a plane perpendicular to a uniform magnetic field is constrained to follow a circular path, the frequency ω_c of which is given by

$$\omega_c = eB/m \quad (1)$$

where B is the magnetic field strength and m/e is the mass-to-charge ratio of the ion. The frequency ω_c is the cyclotron resonance frequency and for a given magnetic field strength, it is characteristic of ions of a given m/e . If an oscillating electric field is applied perpendicular to the direction of the magnetic field, ions of a given m/e will absorb energy when the frequency of this field, ω_1 , is equal to ω_c . This resonance phenomenon is the basis of the detection system in an ICR mass spectrometer: in the instrument, the source region is separated from the analyser region in order to minimize ion trapping and space charge effects. A small electric field is applied perpendicular to the direction of the magnetic field so that the ion follows a cycloidal path, and the overall drift velocity V_D of an ion is given by

$$V_D = l/\tau = E/B \quad (2)$$

where l is the length of the analyser region, τ is the time spent by an ion in this region, and E is the electric field strength.

Fig. 1 shows a standard three-section cell comprised of the source, analyser and ion collector regions. Electrons emitted by a rhenium filament are accelerated through a known variable potential and are collimated by the magnetic field; ions are produced by these electrons along the 2.5 cm of the beam within the cell. All the ions follow a cycloidal path, but space charge repulsion between the similarly charged ions results in a loss of ions in the direction of the magnetic field unless a small trapping potential of the same sign as that of the ions under study is applied to the sides of the cell. This trapping potential is removed in the ion collector region so that ions are collected on the side plates, making it possible to monitor the total ion current. The oscillating electric field is applied to the analyser region: if $\omega_1 \neq \omega_c$, no power absorption occurs and the ion path is as shown schematically in Fig. 2a. For those ions for which $\omega_1 = \omega_c$, energy is absorbed and the ion path for low oscillating field strengths is as shown in Fig. 2b. At very high field strengths, the power absorption is great enough to cause the ions to be lost by striking

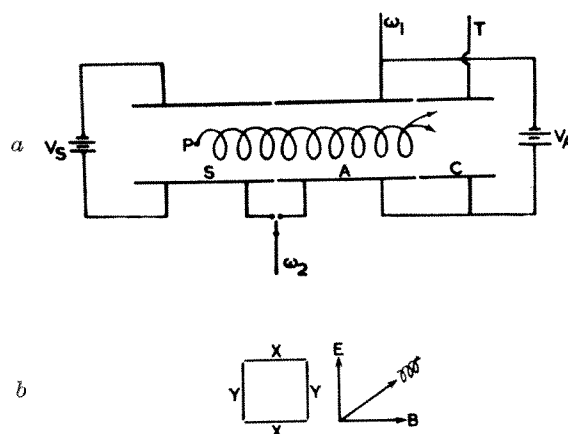


Fig. 1. a, Side view of ICR cell in which the observing oscillator ω_1 is located in the analyser region A, and the irradiating oscillator ω_2 is connected either to the source region S or the analyser region A. The total ion current is measured at T in collector region C. V_S and V_A are the source and analyser drift potentials respectively and P is the electron beam. b, End-on view of the cell showing the directions of the electric and magnetic fields and the ion motion. X and Y are the drift and trapping plates respectively.

the upper and lower plates of the cell, and this can be used to obtain a "total ion current (TIC) spectrum" by monitoring the reduction in total ion current as ions of different m/e are swept out of the analyser region.

Single resonance spectra are usually obtained by holding ω_1 constant and scanning the magnetic field. To improve the signal-to-noise ratio, a phase-sensitive detector is used to measure power absorption. The power absorption can be modulated by using one of several different methods, for example pulsing the source drift field or modulating the electron beam energy. Modulation of the magnetic field gives rise to a derivative spectrum and is preferred for quantitative work because it has least effect on the ion motion and ion density. Typical operating conditions for the observation of Ar^+ ($m/e = 40$) are given in Table 1.

Table 1. TYPICAL OPERATING CONDITIONS IN OBSERVATION OF Ar^+

$m/e = 40$	$B = 8,000$ gauss
$\omega_e = 307$ kHz	Drift field $E = 0.25$ V cm $^{-1}$
$V_D = 3.1 \times 10^3$ cm s $^{-1}$	Analysing region $l = 6.35$ cm
$\tau = 2 \times 10^{-3}$ s	

Radius of non-resonant orbit $r = 0.02$ cm.

Some of the differences between this instrument and the standard analytical mass spectrometer can now be appreciated. The absence of slits results in very high sensitivity so that trap currents are typically 10^{-7} – 10^{-8} A rather than the 10^{-4} A used in analytical instruments. The very long cycloidal path length (typically 10–100 m) means that collision phenomena are important at pressures in the region of 10^{-6} torr so that products of ion-molecule reactions are readily observed. Furthermore, the low drift velocity makes it possible to observe ions milliseconds after formation rather than microseconds as in an analytical instrument. The very low potentials and the magnetic field can easily be reversed, and so the high sensitivity can be utilized in the study of negative ions.

Whereas in an analytical instrument the resolution depends on such factors as beam width and slit width, it depends on quite different instrumental parameters in an ICR mass spectrometer.

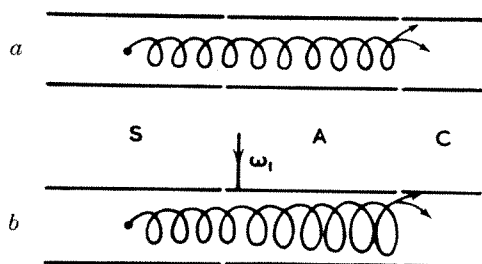


Fig. 2. *a*, Ion motion in the absence of the r.f. field; *b*, ion motion when $\omega_1 = \omega_c$.

Various definitions of resolution are common in mass spectrometry, but in ICR mass spectrometry the resolution $\rho (=M/\Delta M)$ is put equal to $\omega_c/\Delta\omega_{\frac{1}{2}}$, where ω_c is the cyclotron resonance frequency and $\Delta\omega_{\frac{1}{2}}$ is the half-width of the peak in frequency units at half the maximum height. This can be shown to be approximately equal to $2.78/\tau$ where τ is the time during which the ions absorb energy⁵. If τ is the time taken for the ions to pass through the analyser region of the cell,

$$\rho = M/\Delta M = \omega_c/\Delta\omega_{\frac{1}{2}} = \frac{\tau Be}{2.78m} = \frac{lB^2e}{2.78Em} \quad (3)$$

and in typical conditions is in the range 500–2,000. If the ions undergo decomposition during passage through the cell, or if they suffer frequent collisions with neutral species, τ may be significantly shorter than the time required for the ions to pass through the cell, thereby leading to a reduction in resolution.

Two other techniques which have no parallel in conventional mass spectrometry are the double resonance technique⁶ and the ion ejection technique⁷. In almost all cases, the rate constant for an ion-molecule reaction $A^+ + B \rightarrow C^+ + D$ varies with the translational energy of the reactant ion, A^+ , and use can be made of this fact in obtaining double resonance spectra. The magnetic field is adjusted until C^+ ions absorb energy from the observing oscillator of frequency ω_1 .

A second radio frequency field is applied to the source region of the cell, and the frequency of this is varied at constant magnetic field strength. When the frequency of this oscillator, ω_2 , equals the cyclotron resonance frequency of A^+ ions at the particular magnetic field strength in use, A^+ ions absorb energy, causing their translational energy to increase. This will result in a change in the number of C^+ ions observed, provided they are related to A^+ by the reaction we have shown. At a fixed magnetic field strength,

$$m_A/m_C = \omega_1/\omega_2$$

so that by scanning ω_2 , different precursors of C^+ can readily be identified.

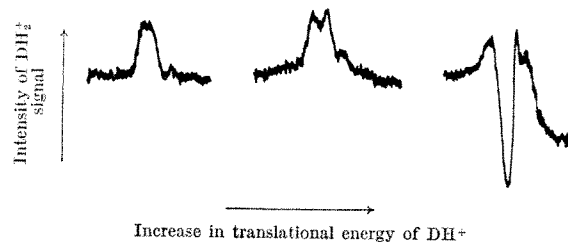
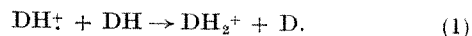


Fig. 3. Double resonance spectra for increasing amplitudes of radio frequency ω_2 for the reaction $\text{DH}^+ + \text{DH} \rightarrow \text{DH}_2^+ + \text{D}$.

If the double resonance signal is due solely to the change in rate constant with increase of translational energy, then the sign of the signal can be interpreted as the sign of the rate of change of the rate constant with the translational energy of the reactant ion, dk/dE_{tr} . But effects arising from ion losses and changes in space charge effects when energy is absorbed may complicate the interpretation.

Fig. 3 shows the double resonance spectrum obtained for the reaction



at increasing amplitudes of the irradiating field. The increase in yield of DH_2^+ ions at low amplitudes suggests that dk/dE_{tr} is positive for this reaction. At higher amplitudes, DH^+ ions gain sufficient energy to be lost from the cell, thereby reducing both the yield of DH_2^+ ions and the total ion current. It is consequently important to monitor the total ion current during an ICDR experiment (unpublished work of G. C. G., A. J. F.-C. and K. R. J.).

Although double resonance spectra allow the identification of ion-molecule reactions, they do not provide a means of determining the relative importance of different reactions leading to the same product ion.

In favourable cases, this can be accomplished by using the ion ejection technique by which ions of a given m/e ratio can be removed selectively from the cell. In addition to the cycloidal motion perpendicular to the direction of the magnetic field, ions undergo simple harmonic motion in the direction of the field in the presence of a trapping potential V_T . The frequency ω_T of this oscillatory motion is given by

$$\omega_T = (4V_T e / md^2)^{1/2} \quad (5)$$

where d is the separation of the trapping plates, but the amplitude is usually insufficient for ions to be lost at the trapping plates. This amplitude, however, can be increased if an oscillating field of frequency ω_T is applied to the trapping plates, so that ions of a given m/e ratio are ejected from the cell. Because ω_T is independent of the magnetic field strength, spectra can be obtained in the normal manner, but in the absence of the ejected ion. By ejecting different ions in turn and observing the variation of the yield of a secondary ion, the relative importance of the ejected ions as precursors of the secondary ion can be determined. The major limitation of the ion ejection technique at present is the very low resolution obtainable, about five, which usually means that more than one ion is wholly or partly ejected.

The quantitative interpretation of ICR spectra is quite complicated and varies with the operating conditions⁸⁻¹⁰, but in the limiting conditions of low ion current density, low conversion and low pressure, the argument is essentially as follows. The peak height is proportional to the power absorption in an ICR spectrum, and at resonance, this is given by

$$A(\omega_c) = Ie^2\epsilon^2\tau^2/8m \quad (6)$$

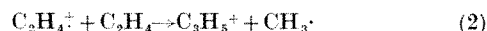
for ions of mass-to-charge ratio m/e , where I is the number entering or leaving the analyser region each second, τ is the time spent in the analyser region and ϵ is the radio frequency field strength. From equations (1) and (2), $\tau \propto m$ for magnetic field scans, so that $A(\omega_c) \propto Im$, if ϵ is constant. Although this argument is approximately correct for primary ions at low conversions, an additional correction is necessary for secondary ions because their yields are approximately proportional to the time which elapses between the formation of primary ions and the average time at which absorption by secondary ions of a given m/e is observed. In a magnetic scan, this time is directly proportional to the mass of the secondary ion, so that for secondary ions, $A(\omega_c) \propto m^2$. In these conditions, therefore, peak heights can be normalized by dividing by m in the case of primary ions and by m^2 in the case of secondary ions. In the case of "TIC spectra", the radio frequency field is used merely to remove ions of a given m/e ratio so that for primary ions no mass correction is necessary, but for secondary ions the signals are normalized by dividing by m in order to correct for the different times allowed for their formation during a magnetic scan. High ion densities, pressures and conversions all serve to complicate the quantitative interpretation of peak heights.

Applications of ICR Mass Spectrometry

One of the chief applications of ICR mass spectrometry has been in the qualitative and semi-quantitative study of ion-molecule reactions in simple systems, using the double resonance and ion ejection techniques to characterize reaction sequences. In normal operating conditions, the translational energies of reactant ions are only slightly in excess of thermal, so that only products

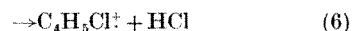
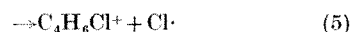
of thermoneutral and exothermic ion-molecule reactions are observed. This contrasts with the ion-repeller field method used in medium and high pressure sources in which translational energies of reactant ions at the exit slit are commonly as high as 2-3 eV. ICR studies of this type will be exemplified by considering ion-molecule reactions in the three systems C_2H_4 , C_2H_3Cl and C_2H_3F .

In the ethylene system^{4,10}, the only primary ion produced at low electron energies is the molecular ion, $C_2H_4^+$. At pressures only a little above 10^{-6} torr, sufficient numbers of collisions occur between these ions and C_2H_4 molecules for the two secondary ions $C_3H_5^+$ and $C_4H_7^+$ to be readily observable. These ions are formed in the reactions

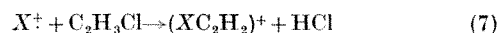


and the observed peak intensities for the two secondary ions lead to $k_2/k_3 =$ approximately 9.5, in good agreement with values obtained by other techniques. Results obtained⁹ with mixtures of C_2H_4 and C_2D_4 showed that the experimental product distribution was very close to that expected on the basis of complete H-D randomization in the collision complex, indicating that the H and D atoms become equivalent in the collision complex. Negative double resonance signals indicated that the rate constants for reactions (2) and (3) decreased as the translational energy of $C_2H_4^+$ increased, whereas positive double resonance signals were obtained for all ions formed in charge-transfer processes. The variations in yields of secondary ions during double resonance experiments suggested that charge transfer processes and the exothermic ion-molecule reactions (2) and (3) occurred competitively at low translational energies of the reactant ion. Typical spectra are shown in Fig. 4.

One of the first systems to be studied by ICR mass spectrometry was chloroethylene², and although only qualitative results were obtained it showed the great potential of the double resonance technique in determining complex reaction sequences. The three major reactions of the molecular ion were found to be



The most common type of reaction of other ions in this system was that analogous to reaction (6), that is



The double resonance technique was used to show that the two Cl atoms become equivalent in the collision complex, for example if $(X^{35}Cl)^+$ reacts with $M^{37}Cl$, the ^{35}Cl atom is not preferentially retained in the charged product, but the two Cl atoms are statistically distributed between the charged and neutral products of the reaction.

Whereas the elimination of HCl from the collision complex is a major reaction path in the chloroethylene system, the corresponding reaction in the fluoroethylene system³ accounts for only about 2 per cent of the reaction products. No reaction analogous to reaction (5) occurs, and the three major reactions of the molecular ion are all

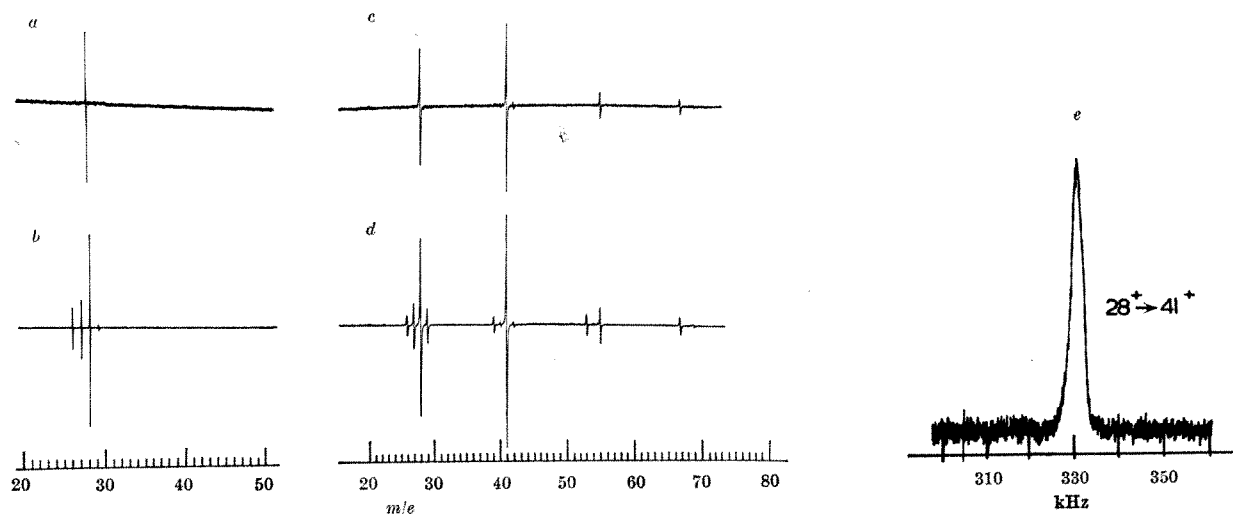
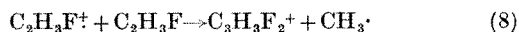


Fig. 4. *a* and *b*, Low pressure spectra of ethylene at 12 eV and 20 eV respectively. *c* and *d*, High pressure spectra of ethylene at 12 eV and 20 eV respectively. *e*, Double resonance spectrum of $m/e = 41^+$; $\omega_1 = 226$ kHz, $\omega_2 = 331$ kHz, showing that the precursor is the molecular ion, $m/e = 28^+$.

analogous to reaction (4) in the chloroethylene system and reaction (2) in the ethylene system:



The relative rate constants for reactions (8), (9) and (10) were found to be 0.59, 0.23 and 0.18 respectively. Reactions of other ions with $\text{C}_2\text{H}_3\text{F}$ are frequently analogous to reaction (7), HF being the neutral species most commonly eliminated.

Reactions which occur in a mixture of ethylene and fluoroethylene illustrate the use of the ion-ejection tech-

nique. A major product is the $\text{C}_3\text{H}_4\text{F}^+$ ion ($m/e = 59$) which may be formed in reaction (9) or in the reactions



The yield of this ion can be observed in the presence of both reactant ions and also when either ion is ejected as is shown in Fig. 5. In this way, it can be shown that reaction (12) is the principal source of $\text{C}_3\text{H}_4\text{F}^+$ ions in the mixture, for the $m/e = 59$ intensity decreases only slightly when the C_2H_4^+ ion is completely ejected (unpublished work of A. J. F-C. and K. R. J.).

Because the translational energies of reactant ions in ICR instruments are close to thermal, product ion distributions may differ from those found using medium pressure sources and the ion-repeller technique. For example, in the propyne system, the formation of C_3H_5^+ in the reaction



is a major process in a medium pressure source at ion exit energies of 1.7 eV (ref. 11), but this is a minor process in an ICR instrument¹². The study of endothermic ion-molecule reactions in ICR instruments, however, is possible by using a technique introduced recently^{13,14} in which the translational energies of reactant ions are increased by subjecting them to a pulse of radio frequency energy at the cyclotron resonance frequency of the ion. A terminal translational energy of several electron volts can be attained during the pulse and reaction occurs subsequently as the ions pass down the drift tube.

A major use of ICR mass spectrometry has been for obtaining data of thermodynamic interest, such as proton and hydrogen affinities, relative gas phase acidities and electron affinities. The proton affinity (P) of a molecule (M) is defined as the negative of the enthalpy change in the reaction



and the hydrogen affinity (H) of an ion (M^+) is similarly defined for the reaction

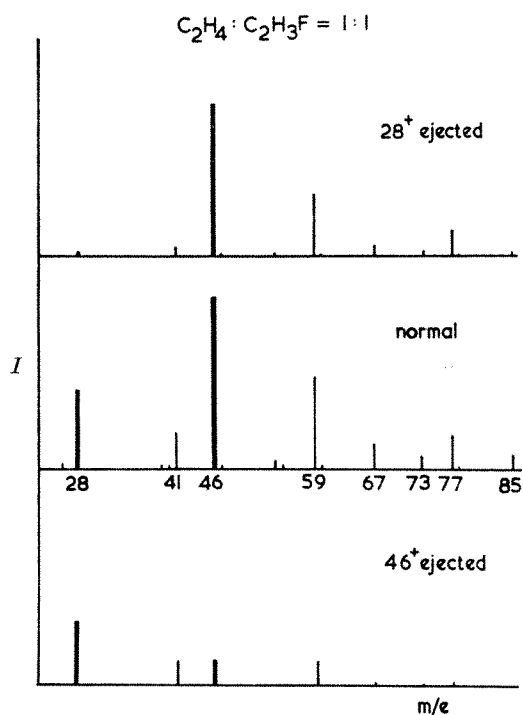
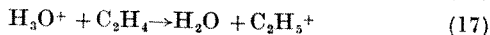
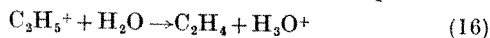
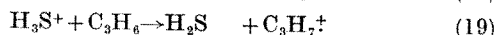
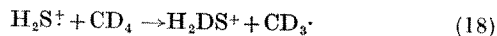


Fig. 5. Ion ejection in a mixture of fluoroethylene and ethylene.

The following two reactions can be observed in an ICR instrument, using the double resonance technique:

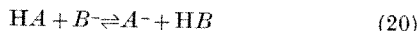


Because the translational energies of the reactant ions are approximately thermal, these two reactions must be thermoneutral or exothermic. When used in conjunction with other thermochemical data, the observation¹⁵ of the occurrence of reactions (16) and (17) leads to $\Delta H_f(\text{H}_3\text{O}^+) = 149 \text{ kcal} = \text{one's mole}^{-1}$, $P(\text{H}_2\text{O}) = 164 \pm 4 \text{ kcal} = \text{one's mole}^{-1}$ and $H(\text{H}_2\text{O}^+) = 141 \pm 4 \text{ kcal} = \text{one's mole}^{-1}$. Similar use of the two reactions

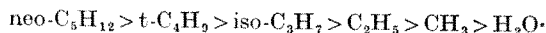


leads to values of $\Delta H_f(\text{H}_3\text{S}^+) = 181\text{--}184 \text{ kcal} = \text{one's mole}^{-1}$, $P(\text{H}_2\text{S}) = 178 \pm 2 \text{ kcal} = \text{one's mole}^{-1}$ and $H(\text{H}_2\text{S}^+) = 106 \pm 2 \text{ kcal} = \text{one's mole}^{-1}$.

The same technique can be used to obtain qualitative information about relative gas-phase acidities¹⁶ of organic compounds such as alcohols and amines by making use of reactions of the type

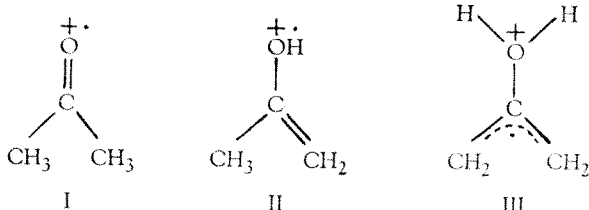


For a particular pair of substances, HA and HB, reaction (19) is usually found to occur in the forward or reverse direction only. For example, if reaction (19) occurs in the forward direction only, the proton affinity of B^- is greater than that of A^- , or in other words, HA is a stronger acid than HB. In the case of the alcohols, it was possible to show that the relative acidities were in the order



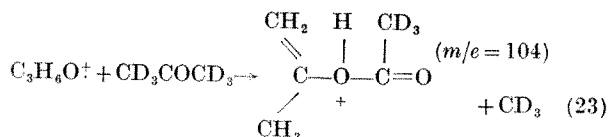
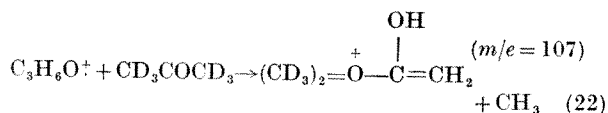
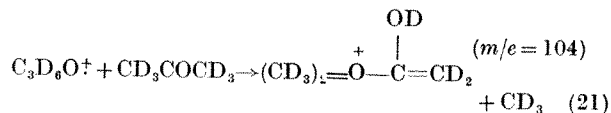
This is the reverse of the order found in aqueous solution and indicates the importance of solvation effects in determining ionic stabilities in solution. If it is assumed that $D(\text{RO}\cdot\text{H})$ is essentially constant in this series, then the relative acidities will reflect the relative electron affinities of the $\text{RO}\cdot$ radicals.

It is frequently found that ions of identical mass and isotopic composition are formed in the mass spectra of different compounds, but from a consideration of the reactions leading to their formation, it is possible that the structures of the ions differ. If it is assumed that ions of different structure undergo different ion-molecule reactions, differences can be detected quite readily by ICR mass spectrometry. This method has been applied recently¹⁷ to the study of reactions of $\text{C}_3\text{H}_6\text{O}^+$ ions produced in different reactions, for which the following structures have been proposed:



It is assumed that the molecular ion of acetone has the structure (I), and there is good evidence that $\text{C}_3\text{H}_6\text{O}^+$ ions produced from 2-hexanone through a McLafferty rearrangement have the structure (II). The $\text{C}_3\text{H}_6\text{O}^+$ ions formed from 5-nonanone by a double McLafferty rearrangement may have the structure (III), although this ion may isomerize to (II) quite readily. By working with a

mixture of CD_3COCD_3 and $\text{n-C}_4\text{H}_7\text{COCH}_3$, it is possible to produce $\text{C}_3\text{D}_6\text{O}^+$ ions of structure (I) and $\text{C}_3\text{H}_6\text{O}^+$ ions of structure (II), thereby allowing one to distinguish many of their reactions. It was found that of the three possible reactions



reaction (21) was the only one which occurs, indicating that this condensation reaction is specific for the keto-form of the ion (I). Several other reactions were found which also distinguish the ions of structures (I) and (II), but no evidence could be found for the independent existence of ions of structure (III), which behaved in all their reactions as if they existed in the enol form, structure (II).

This account indicates the wide range of application of ICR mass spectrometry in the study of the chemistry of ions in the gas phase. Progress has been made recently in the determination of absolute rate constants of ion-molecule reactions in simple systems^{9,10,18,19}, although the analytical expressions are rather complicated if more than one product is formed. Preliminary studies of threshold excitation spectra of neutrals below the ionization limit^{20,21}, and of Penning ionization processes have also been reported²². The open construction of the cell and the absence of discrimination effects at slits are features of the technique which are only just beginning to be explored and it is to be expected that an increasing range of applications for ICR mass spectrometry will be found during the next few years.

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Structure of Lactate Dehydrogenase at 2.8 Å Resolution

by

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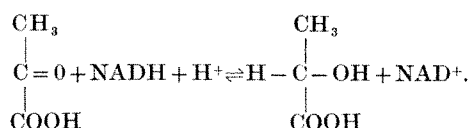
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Electron density distributions for the M₄ isoenzyme of LDH reveal details of the conformation of the subunit, boundaries between the subunits, and features relevant to the binding of coenzyme and substrate.

LACTATE dehydrogenase (EC 1.1.1.27) is an NAD-dependent enzyme catalysing interconversion of lactate and pyruvate in the glycolytic pathway



The enzyme is a tetramer of molecular weight 140,000. Each subunit is capable of binding one molecule of coenzyme and reacts independently^{1,2}. Two subunit types with different electrophoretic mobilities are present in tissue^{3,4}; the M type predominates in skeletal muscle and the H type in heart. Five isoenzymes have been isolated of types H₄, H₃M, H₂M₂, HM₃, and M₄. Enzymatic, immunological, physical and chemical properties are more alike among H or M isoenzymes from different species than between H and M forms of the same species⁵⁻⁸.

An obligatory binding order of coenzyme followed by substrate has been shown for lactate dehydrogenase^{9,10}. A conformational change induced by the AMP portion of the coenzyme permits subsequent binding of nicotinamide¹¹. Isomerization of the enzyme-NADH complex is rate limiting for lactate oxidation at pH 6, whereas for pyruvate reduction the rate limiting steps have been suggested to be the formation of enzyme-NADH-pyruvate at alkaline pH and the interconversion of two enzyme-NAD isomers at pH 6 (ref. 12). The formation and properties of an abortive enzyme-NAD-pyruvate ternary complex have not been studied.

The amino-acid residues involved in the activity of lactate dehydrogenase have not been completely characterized. A dodecapeptide containing a thiol group, the mercuration of which inactivates the enzyme, has been isolated for both M₄ and H₄ isoenzymes of several species^{18,19}. Specific tyrosine and histidine residues have also been shown to be important in the activity of pig H₄ lactate dehydrogenase because their modification partially inactivates the enzyme^{20,21}. A complete sequence of lactate dehydrogenase has not yet been determined. The "essential thiol peptide" has been sequenced for several species and found to be homologous: the sequence for the dogfish M₄ isoenzyme is -Ile-Ile-Gly-Ser-Gly-Cys*-Asn-Leu-Asp-Ser-Ala-Arg²². A thirty-six residue C-terminal peptide of pig H₄ LDH has been determined²¹. The C-terminal dipeptide -Lys-Phe, and the N-terminal peptide of approximate sequence acyl-Thr-Ala-Leu-Lys-Asp-Lys-Leu-(Ala, Thr, Ser)-Ile-Gly-His-Leu-Glu-Pro-Gln-Arg have been reported for the dogfish M₄ isoenzyme (ref. 22 and private communication from N. O. Kaplan and W. S. Allison). Complete studies of amino-acid sequence are being carried out for the pig H₄ and M₄ (private communication from G. Pfeleiderer and K. Mella) and the dogfish M₄ isoenzymes (private communication from N. O. Kaplan and W. S. Allison).

The M₄ isoenzyme of lactate dehydrogenase of dogfish (*Squalus acanthias*) has been shown to crystallize in the

space group F422 with one subunit per asymmetric unit and cell dimensions $a=146.8$ Å, $c=155.35$ Å²³. The centre of the tetramer is at the intersection of three mutually perpendicular 2-fold axes. A 5 Å resolution electron density map has already been described^{24,25}. The structure of the enzyme as obtained from an electron density distribution at 2.8 Å resolution is reported here.

A reduction of crystal symmetry is observed on diffusion into crystals of the apo-enzyme of NAD or any fragment of it that incorporates the AMP moiety. This has been interpreted as a small quaternary structural change such that only the molecular 2-fold axis parallel to c is retained²⁴. The position of the coenzyme in these crystals has been determined at 5 Å resolution where the effects of the space group change are sufficiently small to be neglected²⁵. Crystals of the abortive ternary complex enzyme-NAD-pyruvate have been grown with space group P42₁, $a=95.4$ Å, $c=86.1$ Å and one subunit per asymmetric unit. The tetramer again has 222 symmetry²⁶. An electron density map of this form of the enzyme has been calculated (unpublished results of I. E. S., R. K., M. J. A. and M. G. R.) showing the subunit to be very similar to that of the apo-enzyme except for one part of the chain at the edge of the molecule and near the coenzyme binding site.

Structure Determination

Methods of isomorphous replacement similar to those used for other protein structure determinations were used to obtain the protein electron density distribution. Of the heavy atom derivatives described previously^{24,25} the mercuric chloride, methyl mercuric nitrate (MMN), Baker dimercurial (BDM) and dimercuric acetate (DMA) (partial data) derivatives were used in the 2.8 Å calculations. Low resolution native enzyme data were ob-

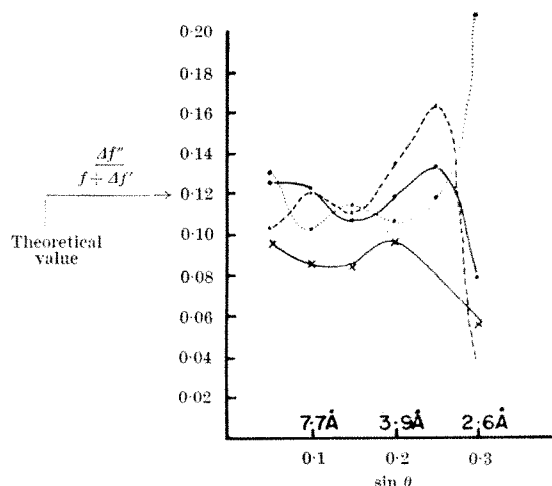


Fig. 1. Variation of anomalous dispersion, as measured by the refined value for $\Delta f''/(f + \Delta f')$, with $\sin \theta$. ●—●, BDM; ○—○, DMA; ×—×, HgCl₂; ●—●, MMN.

tained from a Picker 4-circle card controlled diffractometer. Photographic measurements of the native protein intensities were used between 5 Å and 2.8 Å. These films, obtained by an extension of the scheme described previously²⁴, were scanned on a semi-automatic Joyce Loebel micro-densitometer and absorbance values along each row of reflections were punched on paper tape. Data for heavy atom derivatives, for all terms visible on the native films, were measured on a 4-circle diffractometer. Two Friedel opposites were measured for data to 3.0 Å resolution, and four equivalent reflections, two of type $hk\lambda$ and two of type hkl , were registered between 3.0 Å and 2.8 Å resolution. Integrated intensity values for all reflections in a film plane were computed from the measured absorbances and these L_p corrected sets of data were scaled together in a standard manner²⁷. The diffractometer data were handled as described²⁴ previously with sets of data scaled together with the function

$$F_{\text{scaled}} = b F' - \frac{c}{F'}, \text{ where } F' = F_{\text{meas}} \exp - \frac{B \sin^2 \theta}{\lambda^2}$$

F is a structure amplitude and b and B are scaling parameters. The film set was scaled to the diffractometer sets by the function

$$F_{\text{scaled}} = b F' + \frac{c}{F'}$$

where the term c/F' allows for a constant systematic error on the estimation of the background correction for film intensities.

Refinement of heavy atom parameters and phase angle calculations were carried out as already described²⁴. Heavy atom occupancies of different groups of crystals were refined independently. A somewhat arbitrary weighting function, incorporating the estimated errors for individual reflexions, was used in the phase angle calculation and the refinement. Anomalous dispersion measurements were included in the phase determination, the ratio of the anomalous to real scattering factors being determined as a function of $\sin \theta$ (Fig. 1) for each derivative.

The heavy atom parameters are shown in Table 1 and the mean F and lack of closure errors for all derivatives in Table 2. Fig. 2 shows the distribution of the figure of merit, the calculated heavy atom contribution f_H , and the RMS lack of closure E , with $\sin \theta$ for MMN (the best derivative) and BDM (the poorest).

The "best" Fourier synthesis²⁸ was calculated for 7,650 independent reflexions in sections perpendicular to c at intervals $a/160$, $b/160$, $c/160$ corresponding to 0.92 Å intervals in a and b and 0.97 Å in c . A modified version of a contouring subroutine (written by Zisk and Brenner at MIT) was used to produce points of equal electron density which were joined by spline-like curves²⁹ to produce contoured sections. These sections were drawn on a Calcomp plotter, photographed and redrawn on glass at the scale of 2 cm = 1 Å for model building using a Richards optical comparator³⁰ and Kendrew model parts (manufactured by the Cambridge Repetition Engineers).

The electron density map has been interpreted in terms of a single polypeptide chain of some 311 residues

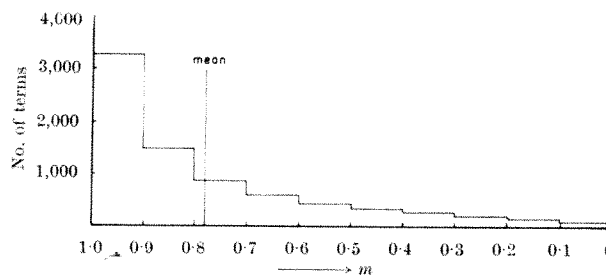


Fig. 2. Figure of merit distribution

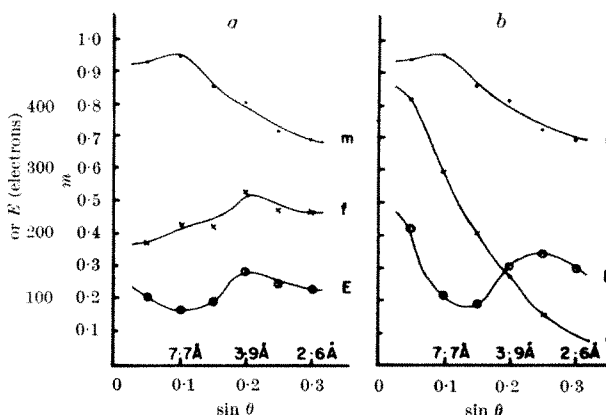


Fig. 3. Various indicators of error plotted as a function of $\sin \theta$ for (a) the best (MMN) and (b) the poorest (BDM) compound.

whereas the most recent amino-acid analysis suggests approximately 326 residues²². Because a complete amino-acid sequence is not yet available and the electron density distribution at 2.8 Å does not allow unambiguous distinction between different amino-acids, the side chains were fitted only as far as their β carbons, except for the eighteen residues at the amino end, thirteen in the essential thiol peptide and two at the carboxyl end. Fig. 4, map sections 30 to 40/160c, illustrates the fit of the electron density to the polypeptide backbone in the region of the anti-parallel ribbon, as well as the known residues near the amino terminus. The contrast between solvent and protein was found to be excellent. There is only one major uncertainty of chain folding, and this occurs about twenty residues from the N-terminus, where the density used is very close to a 2-fold axis and either of two junctions may be made.

The Molecule of Lactate Dehydrogenase

The conformation of the lactate dehydrogenase subunit, viewed from the molecular centre looking down c , is illustrated in Fig. 5. Features of secondary and tertiary structure, including the nomenclature used in the text, are summarized in Table 3. About 24 per cent of the residues are in α -helical conformation and a further 10–15 per cent in β structure, the latter being a well defined parallel pleated sheet resembling that found in subtilisin³¹ and less well defined anti-parallel pleated ribbon. A further sixteen residues are involved in single

Table 1. HEAVY ATOM PARAMETERS

Sites	Compounds	Positional parameters (fractional coordinates)			Occupancies in electrons Crystal group								Temperature factors in Å ²
		<i>x</i>	<i>y</i>	<i>z</i>	1	2	3	4	5	6	7	8	
A	HgCl ₂	0.141	0.140	0.230	75	43	60	67	83	117	175	87	67.3
	DMA	0.136	0.138	0.228	113	94	86						69.9
	MMN	0.144	0.142	0.229	35	5	18	13	57	46	41		18.1
	BDM	0.146	0.143	0.225	117	101	132						81.3
A'	DMA	0.121	0.119	0.233	69	51	43						58.7
A''	BDM	0.156	0.176	0.215	31	25	24						26.1
B	BDM	0.104	0.296	0.135	6	52	26						57.5
	HgCl ₂	0.395	0.204	0.135	115	64	107	146	98	106	106	109	3.7
D	MMN	0.407	0.209	0.138	39	37	36	52	44	42	46		0.9
	MMN	0.086	0.219	0.200	65	52	97	121	87	98	74		10.8

turns of 3_{10} helix. Except for twenty residues at the N-terminus, the subunit is globular; it may be viewed as two halves separated by a considerable cleft with three connecting chains. The left hand portion, consisting principally of the N-terminal 130 residues, has the greater proportion of regular secondary structure. It contains four α helices (42 residues) and a stretch of parallel pleated sheet (23 residues). The adenine end of the coenzyme binding site is within this part of the subunit; the nicotinamide end is within the cleft. The right half, with less obvious secondary structure, contains the "essential thiol peptide" which also borders the cleft. Residues 194-209 form a winglike feature which is a part of the boundary of the right half with solvent. At the front of the subunit the two halves are joined by an imperfect anti-parallel pleated ribbon which also forms the subunit boundary. Helix H (289-300), slightly separated from the rest of the subunit, is close to the C terminus. The last eight residues are an extended chain with density consistent with a phenylalanine at the end.

Table 2. 2.8 Å REFINEMENT: ERROR ANALYSIS

Minimum spacing (Å)	15.42	7.71	5.14	3.86	3.08	2.57
Sin θ	0.050	0.10	0.15	0.20	0.25	0.30
Mean F_F	1055	1339	892	1275	1063	866
HgCl ₂						
RMS closure E	174	136	173	141	154	109
RMS small f	397	377	367	288	327	327
RMS differences	389	313	310	244	270	224
RMS F errors	67	84	125	66	84	89
R modulus	40.9	34.1	42.5	43.8	37.5	26.3
R weighted	13.8	10.7	13.4	23.1	16.5	9.0
Number per zone n	78	505	1268	1915	2499	1237
DMA						
RMS closure E	129	58	67	91	135	205
RMS small f	459	294	182	131	97	68
RMS differences	455	235	152	141	162	214
RMS F errors	45	49	48	52	63	85
R modulus	25.7	18.7	34.7	67.9	130.1	262.2
R weighted	5.1	3.3	8.4	43.5	164.1	521.6
Number per zone n	52	429	947	1575	981	77
MMN						
RMS closure E	106	88	99	147	127	119
RMS small f	195	236	227	257	233	222
RMS differences	198	209	197	230	205	180
RMS F errors	41	50	47	74	70	77
R modulus	48.4	34.8	38.9	49.5	45.2	45.4
R weighted	18.6	11.3	14.5	24.5	24.5	21.7
Number per zone n	57	440	974	1889	2495	1193
BDM						
RMS closure E	213	109	96	154	170	148
RMS small f	401	297	204	140	80	49
RMS differences	456	255	183	183	179	154
RMS F errors	58	63	72	86	105	107
R modulus	48.3	35.5	43.0	100.2	195.7	262.6
R weighted	26.3	11.5	15.9	69.1	361.8	751.0
Number per zone n	77	505	1268	1824	1843	1037

Lack of closure and structure amplitudes are in electrons.

F_P = native protein structure amplitude

F_{PH} = heavy atom derivative structure amplitude

f_s = calculated heavy atom contribution

n = number of terms

RMS closure $E = (\sum (F_{PH} - |F_P + f_H|)^2/n)^{1/2}$

RMS small $f = (\sum f_H^2/n)^{1/2}$

RMS differences $= (\sum (F_{PH} - F_P)^2/n)^{1/2}$

RMS F errors $= (\sum \sigma_{PH}^2/n)^{1/2}$

σ_{PH} = experimental error in F estimated from counting statistics

R modulus $= \sum |F_{PH} - |F_P + f_H|| / \sum f_H$

R weighted $= \sum \omega (F_{PH} - |F_P + f_H|)^2 / \sum \omega f_H^2$

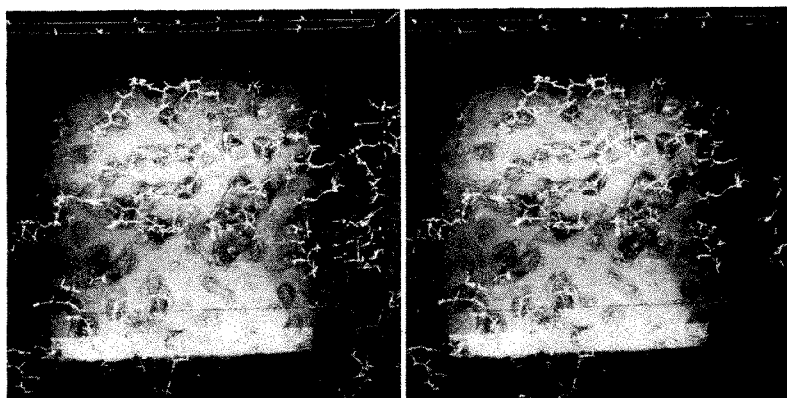
ω = weight used for term in least squares refinement

Residue	Secondary structure	Comment
1	↓ α -helix A	↓ N terminal ambiguity
6		
20		
22	↓ parallel pleated sheet, first pass	
26		
32	↓ α -helix B	
40		
45	↓ parallel pleated sheet, second pass	
48		
55	↓ α -helix C	
61		
70	↓ parallel pleated sheet, third pass	
72		
79	↓ parallel pleated sheet, fourth pass	
86		↓ Loop involved in tertiary structural change in abortive ternary complex
95	↓ α -helix D	
106		
109	↓ α -helix E	
118		
121	↓ parallel pleated sheet, fifth pass	
123		
144		
153	↓ α -helix F	↓ Essential thiol peptide
155		
156		↓ "Wing"
194		
205		
229	↓ α -helix G	
242		
245	↓ anti-parallel ribbon, first strand	
261		
262	↓ anti-parallel ribbon, second strand	
276		
277	↓ anti-parallel ribbon, third strand	
283		
289	↓ α -helix H	
300		
311		C terminal Phe

The two possible positions of the N-terminal 20 residues relative to two monomers are shown in Fig. 6. Residues 1-18 occupy symmetry equivalent electron density in either interpretation. The proposed sequence for these residues²² fits the density adequately. The preferred orientation has been chosen in spite of its unusual conformation because the electron density distribution is more continuous. These alternatives have interesting implications. In the position preferred, residues 1-18 of the first subunit interact with a second subunit formed by rotation about the 2-fold axis parallel to c . The helix A wraps around the far side of the second subunit. The less preferred interpretation involves residues 7-18 folding back over the first subunit; helix A then projects in front of the rest of the subunit and is in contact with a third subunit formed by rotation of the first about the 2-fold axis parallel to a . The preferred orientation involves many more contacts between subunits. These seem to be both hydrogen bonding and hydrophobic interactions, but in the absence of a complete sequence, no more detailed interpretation of these contacts can be made.

Residues 22-26 form the central strand of the parallel pleated sheet; the chain then passes beneath the sheet forming a nine residue helix B (32-40) to the second strand (45-48) and beneath again through helix C (55-72). The chain then passes above the sheet and a fourth strand forms next to the central one (78-86). A loop

Fig. 4. Stereo view of LDH skeletal model optically superimposed on sections $z=30$ to $40/160c$, showing the fit of the electron density to the polypeptide backbone (in centre) and known amino-acid residues (at top).



across the back of the subunit, including helix D (95–106), (12 residues) forms a lip of the central cleft. Helix D (10 residues, 109–118) above the sheet, follows immediately and the fifth strand of sheet (121–123) next to the cleft is then formed. The hydrogen bonding within the sheet is shown in Fig. 7. Residues immediately following the three central strands of the sheet are involved in contacts to the adenine, adenine ribose, phosphates and nicotinamide ribose, while the C-terminal residue of helix D is in contact with the adenine (Fig. 8). Caution should be observed, however, in considering which residues are involved in coenzyme binding, for the reduc-

tion in crystal symmetry and probable conformation change occurs when any fragment of the dinucleotide larger than adenosine is diffused into the crystals.^{11,24}

The "essential thiol peptide" has been identified because residue 149 is the ligand for the B site of mercury substitution in the HgCl₂ and MMN derivatives^{24,25}. The known sequence of this peptide has been fitted to the electron density satisfactorily and its conformation is shown in Fig. 9. It bounds the central cleft opposite the coenzyme binding site. The sulphydryl is separated from the cavity by this part of the main chain, but both serine 147 and asparagine 150 point into the cavity

Fig. 5. α -Carbon positions for one subunit viewed along c from the molecular centre, with preferred positions of N-terminal residues.

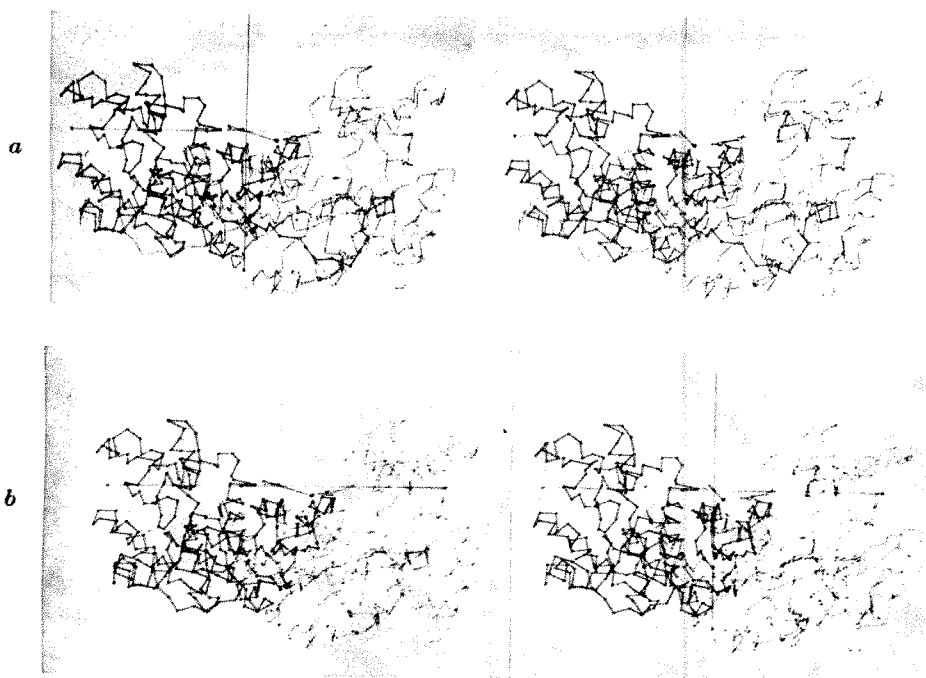
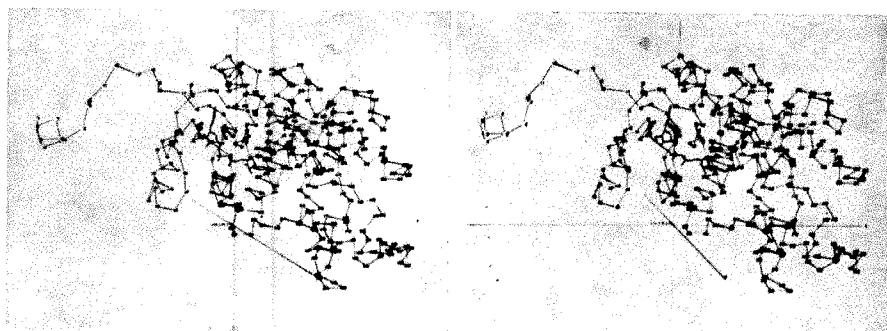
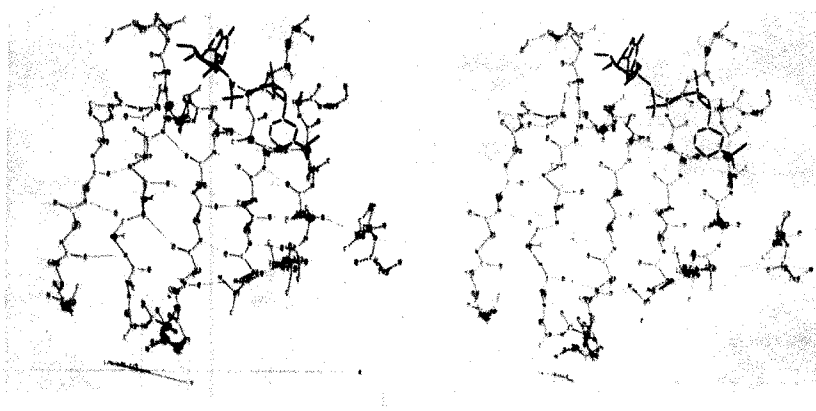


Fig. 6. Schematic view of two subunits related by a 2-fold rotation about the molecular axis parallel to y , showing (a) the preferred orientation of residues 1–20, and (b) the less preferred interpretation.

Fig. 7. Five-stranded parallel sheet and coenzyme. β -Carbon positions and hydrogen bonding of sheet are indicated.



above and below the nicotinamide and about 6 Å away. The main chain again approaches the central cleft at residue 181. It is interesting that the essential thiol of pig glyceraldehyde 3-phosphate dehydrogenase is 149 and the essential lysine is 183^{32,33}. A chain conformation similar to that of dogfish M₁ LDH would enable an active centre including these residues to be formed.

The central cleft is shown in Fig. 8. Its opening to solvent is made by the loop (86-109) joining the fourth strand of parallel sheet and helix E. A comparison of the apo-enzyme and the abortive ternary complex at 5 Å resolution (Fig. 10) shows the major difference between them to be the conformation in this region. In the ternary complex the loop moves about 12 Å so as to close the

entrance to the cleft and covers the nicotinamide end of the coenzyme. A rather isolated peak found in the 5 Å map of the apo-enzyme and interpreted as a solvent ion in the 2.8 Å map, is considerably enlarged in the 5 Å map of the abortive ternary complex. This peak is near asparagine-150, serine-147, and the nicotinamide of the coenzyme. Whether this represents a substrate binding site might be resolved when a high resolution map of the ternary complex has been calculated.

The parts of the subunit which form boundaries may be noted, although it is difficult to make a detailed analysis of contacts in the absence of a complete sequence. Subunits related by the 2-fold axis parallel to *c* have the helices C, B, and G of one in contact with G, B, and C

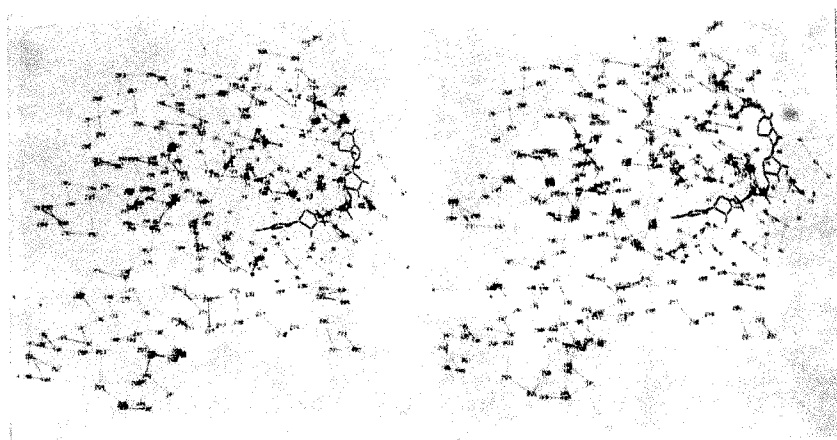


Fig. 8. View from molecular boundary showing the coenzyme and central cavity.

Fig. 9. Arrangement of thirteen residues of the "essential thiol" peptide and their spatial relationship to the nicotinamide end of the coenzyme.

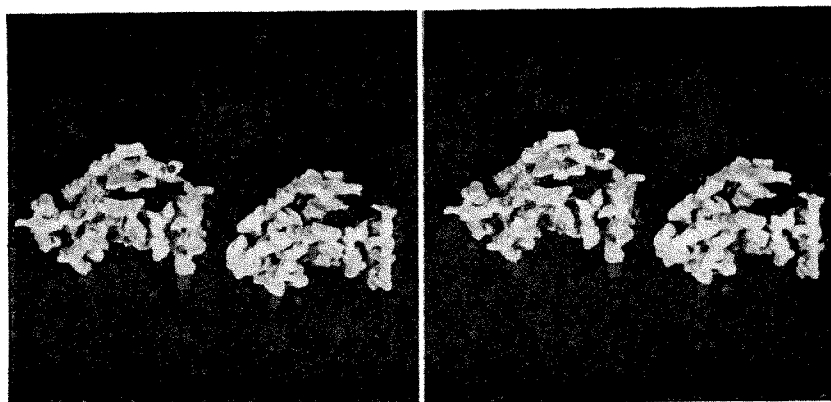
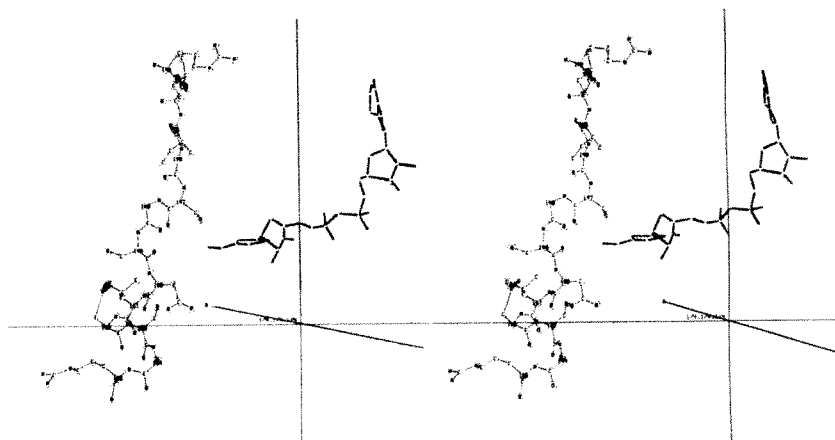


Fig. 10. The 5.0 Å electron density models of the abortive ternary complex (left) and the apo-enzyme with the coenzyme placed into it (right). The coenzyme is shaded.

of the other, respectively. The N-terminal helix A makes contacts with the winglike feature of the subunit related to it by the molecular axis parallel to *c*. The subunits related by the axes parallel to *a* and *b* have in contact mainly the anti-parallel pleated sheet and some random coil at residues 160–170. Close to these residues all four subunits are in contact. The coenzyme binding site and the region expected to bind substrate are both wholly within one subunit. The closest approach between adjacent coenzymes is 19 Å, while the nicotinamide ends are as much as 26 Å apart. The elucidation of the structure of the enzyme-coenzyme complex and of the abortive ternary complex may throw light on the importance of a tetrameric molecule.

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Mechanism of Pyruvate Carboxylase Formation from the Apo-Enzyme and Biotin in a Thermophilic Bacillus

by

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Pyruvate carboxylase is formed by enzyme-catalysed attachment of biotin to an inactive protein precursor. Acetyl-CoA and aspartate allosterically influence this reaction.

THE enzyme pyruvate carboxylase is synthesized by the addition of biotin to its inactive protein precursor, apo-pyruvate carboxylase. This reaction is catalysed by holoenzyme synthetase. When grown without a supply of biotin, a strain of thermophilic bacillus synthesizes large amounts both of the inactive apo-pyruvate carboxylase and of the holoenzyme synthetase. Pyruvate carboxylase activity appears rapidly when biotin is added to these cells, in the absence of protein synthesis. The reconstitution of the enzyme activity in cell-free extracts demands acetyl-CoA as well as Mg^{2+} , biotin and ATP¹. Neither the need for acetyl-CoA, nor the effect of L-aspartate in antagonizing its action, has been observed with other carboxylating systems^{2–9}.

The apo-enzyme can be purified from cultures of the bacillus, (a starch-negative variant of *Bacillus stearothermophilus*¹⁰, previously referred to as *B. coagulans* sp.¹), which have been grown on succinate medium, by a procedure similar to that described¹¹ for the purification of the active pyruvate carboxylase. This involves growth of the organism¹² at 55° C, preparation of extracts of the washed cells by digestion with lysozyme, and repeated

fractionation with ammonium sulphate. A fraction containing both the apo-enzyme and the holoenzyme synthetase can then be resolved into the two protein components by chromatography on 'Sephadex G-200' as illustrated by the elution profiles of Fig. 1, which demonstrate that the apo-enzyme is eluted well before the holoenzyme synthetase. This experiment was designed primarily to demonstrate the effect of acetyl-CoA on the incorporation of ¹⁴C-biotin into the apo-enzyme. When acetyl-CoA was not included in the incubation mixture, there was very little incorporation of (+)-¹⁴C biotin into the apo-protein and virtually no formation of pyruvate carboxylase activity (Fig. 1a). When the experiment was repeated with the sole difference that 2.5 μmoles of acetyl-CoA were also included in the incubation mixture, the results obtained were markedly different. Thus Fig. 1b shows a large peak of radioactivity associated with a protein, which possessed pyruvate carboxylase activity, was eluted at the same place as that occupied in Fig. 1a by the apo-protein, and was well separated from the synthetase. Clearly, the inclusion of acetyl-CoA in the incubation system leads to the reconstitution of the active

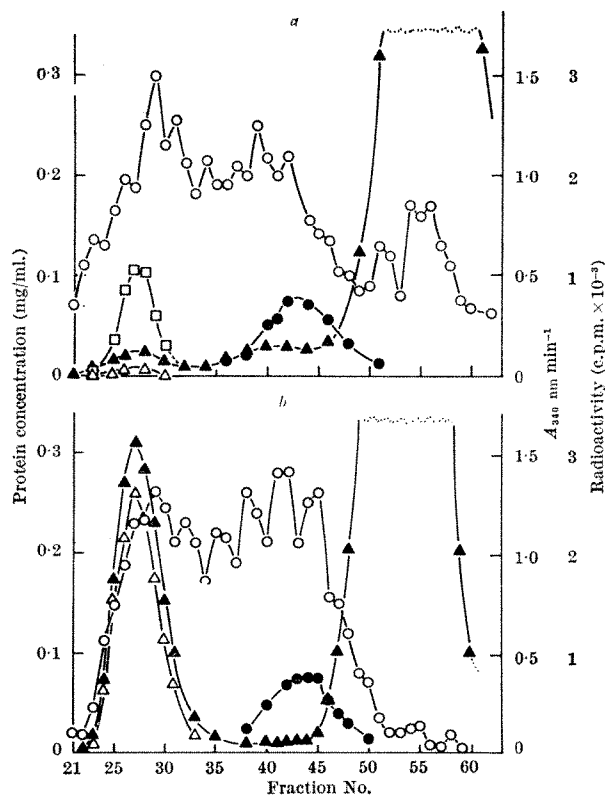


Fig. 1. *a*, Requirement for acetyl-coenzyme A in the formation of active pyruvate carboxylase from the apo-enzyme and ^{14}C -biotin. The purified apo-protein (2.2 mg) and holoenzyme synthetase (3.1 mg) obtained after 'Sephadex G-200' chromatography were mixed with 12.5 μmoles of MgCl_2 , 3.1 μmoles of ATP, 0.17 μmoles of (+)- ^{14}C -biotin (containing 10 μCi of isotope) and 37.5 μmoles of Tris-HCl buffer (pH 7.6), in a final volume of 7 ml. The mixture was incubated for 30 min at 45°C , cooled to 0°C , 20 μmoles of EDTA were added and the proteins were precipitated with 20 ml of saturated ammonium sulphate. The precipitate was dissolved in 0.5 ml of 50 mM-Tris-HCl buffer (pH 7.6), containing 1 mM EDTA and 0.4 M ammonium sulphate, and the solution was applied to a column of 'Sephadex G-200' (1.7 \times 30 cm) equilibrated with this buffer; the proteins were then eluted with the same buffer. Fractions of 1 ml. were collected and assayed for protein, radioactivity, and the activities of pyruvate carboxylase, the apo-enzyme, and holoenzyme synthetase. Protein (O) was determined spectrophotometrically¹³ and radioactivity (Δ) was measured in a Tracerlab scintillation spectrometer after mixing samples (0.5 ml.) with 5 ml. of Bray's fluid¹⁴. Holoenzyme synthetase (●) was assayed spectrophotometrically¹. For the assay of apopyruvate carboxylase (□) or holoenzyme synthetase (●) the sample was incubated in a mixture (0.3 ml.) containing (in μmoles) Tris-HCl (pH 7.6), 15; MgCl_2 , 0.5; acetyl-coenzyme A, 0.1; (+)-biotin, 0.07, and an excess of synthetase or apo-enzyme as appropriate. After incubation at 45°C for 15 min, the reaction was stopped by placing the tubes in an ice bath and adding 1 μmole of EDTA. Portions (0.2 ml.) of these mixtures were withdrawn and the holoenzyme synthetase formed was determined spectrophotometrically¹ by measuring the rate of decrease in extinction at 340 nm concomitant with the oxidation of NADH, in the presence of malate dehydrogenase, by the oxaloacetate synthesized in the pyruvate carboxylase reaction. One unit of apopyruvate carboxylase is defined as that amount of the apo-enzyme which, on total conversion, yields one unit of holoenzyme, which catalyses the formation of one μmole of oxaloacetate per min at the temperature of assay (45°C). One unit of synthetase is defined as that amount of the enzyme which catalyses the conversion of one unit of the apo-enzyme to the holoenzyme per min at 45°C . *b*, Protein (O), radioactivity (Δ), holoenzyme synthetase (●) and holoenzyme synthetase (●) were assayed as in *a*. Conditions were as in *a* except that 2.5 μmoles of acetyl-CoA were included in the initial incubation mixture.

enzyme from the apo-protein, and to the attachment of biotin to that protein.

As shown in Table 1, this reconstitution reaction required the two protein components (apo-enzyme and synthetase), a divalent cation (Mg^{2+} or Mn^{2+}), (+)-biotin, ATP and acetyl-CoA. In this system, with an excess of apo-carboxylase, formation of active enzyme was linear with respect to time of incubation (15–20 min) and to the amount of synthetase protein. With an excess of synthetase protein and an appropriate incubation period (15 min), the formation of active enzyme was proportional to the amounts of apo-enzyme added. Acetyl-CoA was required for pyruvate carboxylase formation even at 30°C , at which temperature both the

apo-enzyme and the holoenzyme synthetase were completely stable for the times of incubation used. This suggests that acetyl-CoA acts as an allosteric effector of the reconstitution process rather than as a stabilizing agent for one or other of the protein components of the reaction system.

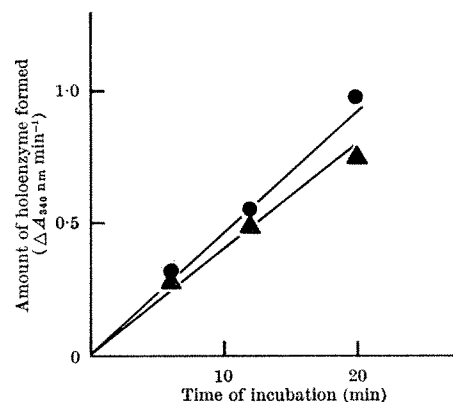


Fig. 2. Replacement of (+)-biotin, ATP and MgCl_2 by biotinyl-AMP for the formation of pyruvate carboxylase. The reaction mixture with biotin-ATP- MgCl_2 (●) was as described in the legend to Fig. 1a, for the assay of apopyruvate carboxylase and holoenzyme synthetase, with 0.35 mg of apopyruvate carboxylase and 0.41 mg of synthetase. The reaction mixture with biotinyl-AMP (Δ) contained (in μmoles) in a final volume of 0.3 ml.: Tris-HCl buffer (pH 7.6) 15; acetyl-CoA, 0.1; biotinyl-AMP, 0.067; and apopyruvate carboxylase and synthetase as above. Following incubation at 45°C as indicated, the holoenzyme synthetase formed was determined.

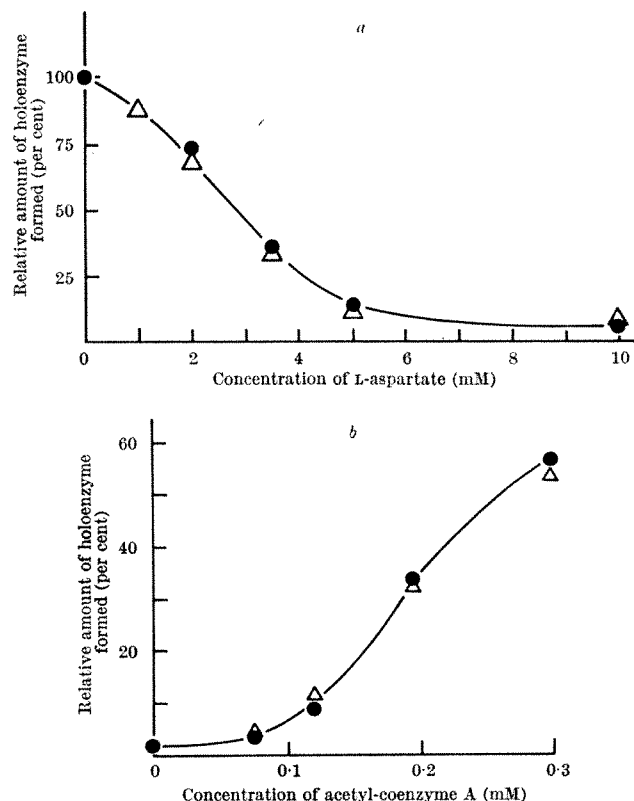


Fig. 3. Effect of acetyl-CoA and L-aspartate on the synthesis of holoenzyme synthetase. *a*, Holoenzyme synthesized at different L-aspartate concentrations with a fixed concentration of acetyl-CoA (75 μM) was measured in the reaction systems described in the legend to Fig. 1 and expressed as per cent of the active enzyme formed in the absence of L-aspartate. In the absence of L-aspartate, the amount of active enzyme formed was 0.155 unit with biotin-ATP- MgCl_2 , and 0.092 unit with biotinyl-AMP. ●, (+)-biotin-ATP- MgCl_2 system; Δ, biotinyl-AMP system. *b*, Holoenzyme synthesized at different acetyl-CoA concentrations and at a fixed concentration of L-aspartate (10 mM) was determined and expressed as per cent active enzyme formed at an acetyl-CoA concentration of 75 mM in the absence of L-aspartate. The 100 per cent values were 0.191 unit of holoenzyme for the system with biotin-ATP- MgCl_2 , and 0.095 unit for the system with biotinyl-AMP. ●, (+)-biotin-ATP- MgCl_2 system; Δ, biotinyl-AMP system.

Table 1. REQUIREMENTS FOR HOLOPYRUVATE CARBOXYLASE SYNTHESIS FROM BIOTIN AND ATP

	Pyruvate carboxylase synthesized (per cent of complete)
Complete	100
Reduced glutathione (1 μ mole) added	91
MgCl ₂ omitted	15
MgCl ₂ omitted; MnCl ₂ (0.5 μ mole) added	92
Acetyl-coenzyme A omitted	9
Biotin omitted	0
ATP omitted	9
Apopyruvate carboxylase omitted	0
Synthetase omitted	0

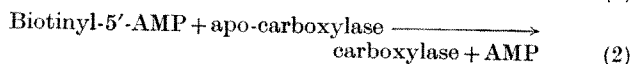
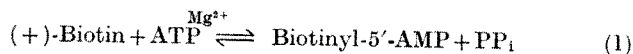
The complete system was the one described in legend to Fig. 1; 64 μ g of apopyruvate carboxylase and 78 μ g of synthetase were used. Additions and omissions were made as indicated. The amount of pyruvate carboxylase synthesized in the complete system (taken as 100 per cent) was 0.168 unit.

Pyruvate carboxylase was reconstituted also in mixtures of the purified apo-enzyme and holoenzyme synthetase when synthetic (+)-biotinyl-5'-AMP (biotinyladenylate) was used in place of biotin and ATP: the adenylate derivative was almost as efficient as (+)-biotin and ATP in this process (Fig. 2). Again, the active enzyme was formed only if both protein components, biotinyl-AMP and acetyl-CoA were present; in the absence of acetyl-CoA, the yield of active enzyme was reduced by 94 per cent. Unlike the system described in Table 1, Mg²⁺ was not required for the formation of pyruvate carboxylase when biotinyl-AMP was the biotin donor, and EDTA did not inhibit the reaction.

An inhibitory action of L-aspartate on the synthesis of active enzyme, was demonstrated in our earlier studies with crude extracts¹. This has now been observed also in the purified, resolved system. Fig. 3 shows the effect of L-aspartate (a) or acetyl-CoA (b) on the formation of active enzyme. In either case, the effect was similar whether biotinyladenylate, or biotin and ATP were supplied. The two sets of curves show the mutual antagonism between acetyl-CoA and L-aspartate and their pronounced sigmoidicity suggests strongly that these compounds are exerting an allosteric effect on the formation of pyruvate carboxylase.

The resolution of the pyruvate carboxylase reconstitution system into the apo-enzyme and the holoenzyme synthetase, and the efficiency with which biotinyladenylate substitutes for biotin and ATP in it, underline the similarity of the mechanisms of synthesis of biotin-dependent carboxylases from their apo-enzymes and biotin. Formation of active propionyl-coenzyme A carboxylase⁴, methylmalonyl-coenzyme A-oxaloacetate trans-

carboxylase⁵ and acetyl-coenzyme A carboxylase⁶ takes place in two steps, catalysed by holoenzyme synthetases that are not species specific. By analogy, the synthesis of active pyruvate carboxylase in our system can be represented as



The target of action of acetyl-CoA in this sequence of reactions is not certain. Although experiments with biotinyladenylate prove that acetyl-CoA is essential for the second step, the effect could be on either the apo-carboxylase or on the synthetase, or both. Because acetyl-CoA is required for the activity of the holoenzyme¹², the apoprotein is the most likely point of action of the activator; but we cannot yet rule out a possible effect of acetyl-CoA on the synthetase that effects both the synthesis of biotinyl-5'-AMP and its reaction with the apo-enzyme.

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Control of Synthesis and Wastage of Ribosomal RNA in Lymphocytes

by

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Phytohaemagglutinin stimulates the synthesis of the 45S ribosomal precursor in lymphocytes without concomitant protein synthesis; it also decreases the loss of newly synthesized 18S RNA that normally takes place in these cells at rest.

STIMULATION of RNA synthesis in human lymphocytes by phytohaemagglutinin (PHA)¹⁻⁴ causes an increase in the synthesis of 45S RNA which is disproportionately large relative to the stimulation of overall RNA synthesis². We have shown that, in the resting lymphocyte, approximately half of the newly synthesized 18S rRNA molecules are rapidly degraded, apparently without ever entering the cytoplasm^{5,6}. This is detected by a disparity in the labelling ratio between 28S and 18S rRNAs, which should be synthesized, and labelled, in equimolar amounts

according to our present understanding of the mode of synthesis of rRNA. Both forms of rRNA derive from a single 45S precursor molecule, implying coordinate production of the two forms⁷⁻¹⁰. The excess new 28S rRNA molecules remaining after rapid degradation of 18S rRNA are also degraded, but only after a lag period, thereby permitting the detection of the early loss of 18S molecules⁶. The wastage of rRNA seen in resting lymphocytes is promptly reduced by PHA⁶.

Thus PHA seems to act in at least two different ways

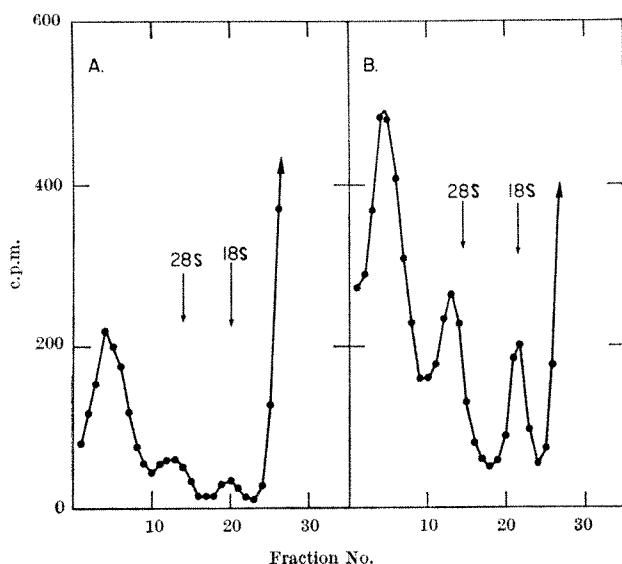


Fig. 1. Early effect of PHA on 45S RNA synthesis. 5×10^7 lymphocytes were incubated in Eagle's minimal essential medium, deficient in methionine, at a density of 2×10^6 /ml. [$\text{methyl-}^3\text{H}$]Methionine (20 $\mu\text{Ci}/\text{ml}$, 4.7 Ci/mmol, Schwarz Biosearch) was added for a 1 h labelling period. RNA was then extracted with phenol-sodium dodecyl sulphate at 40°C (ref. 12) and sedimented on sucrose gradients (8–25 per cent) for 17 h at 22,000 r.p.m. (Spinco 'SW-41' rotor). Serial gradient fractions were collected from the bottom of the tube and acid insoluble radioactivity in each fraction was determined. Absorbance markers were provided by rRNA extracted from rat liver and added during the extraction procedure (arrows). Direction of sedimentation is right to left. A, Resting lymphocytes; B, PHA (2.5 $\mu\text{g}/\text{ml}$, Burroughs-Wellcome) added 1.5 h before labelling.

to provide the stimulated lymphocyte with the means of increasing rRNA production: by increasing initial synthesis, and by reducing wastage of newly produced molecules. It is not clear, however, whether these effects are independent actions of PHA on different biochemical events, or are interdependent effects, one occurring as a consequence of the other. I have now resolved these phenomena, which I conclude to be independent of one another and to have different biochemical characteristics.

Human peripheral blood lymphocytes were used in these experiments¹¹. Newly synthesized rRNA was labelled with ^3H -methyl groups from [$\text{methyl-}^3\text{H}$]methionine⁶ and extracted with phenol and sodium dodecyl sulphate¹². Heterogeneous forms of RNA are not labelled by this procedure, whereas rRNA is specifically labelled^{13–15}.

The early stimulation of 45S RNA synthesis by PHA has been demonstrated previously using ^3H -uridine labelling². In Fig. 1 this stimulation is confirmed using [$\text{methyl-}^3\text{H}$]methionine as a precursor. Pre-treatment of resting lymphocytes with cycloheximide, in a dose which eliminated 98 per cent of protein synthesis, caused a fall in the rate of 45S synthesis, but subsequent addition of PHA still produced a rise in 45S RNA labelling (Fig. 2). This was not detectable in earlier studies using ^3H -uridine labelling because of interference by the large amount of radioactivity entering heterogeneous forms of RNA². It is evident, however, that synthesis of 45S RNA may be increased, to some extent, without a requirement for new protein synthesis.

Several investigators, using various eukaryotic systems, have reported that abolition of protein synthesis with cycloheximide results in a marked fall in production of new 18S rRNA molecules^{9,16–19}. This is apparently the result of the rapid degradation of newly formed 18S molecules when concomitant protein synthesis

is prevented. We have observed the same effect in lymphocytes, both resting and growing, and in Burkitt lymphoma cells. This effect is illustrated as follows (Fig. 3B, D).

The untreated, resting lymphocyte, in a very similar manner, has been shown to degrade a large portion of its newly synthesized 18S rRNA^{5,6}. This is illustrated in Fig. 3A, where the ratio between radioactivity incorporated into 28S and 18S rRNAs during a 2 h pulse is 3.8. Equimolar production of 28S and 18S rRNAs, which is anticipated from the mode of rRNA synthesis, would give a 28S : 18S labelling ratio of 1.4–1.5, based on the molecular weights of the two forms and their characteristic degrees of methylation³. The observed 28S : 18S ratio implies the loss of more than 60 per cent of newly synthesized 18S rRNA molecules. Treatment of the cells with cycloheximide, before labelling, results in an even greater 28S : 18S disparity, implying still greater degradation of newly synthesized 18S rRNA (Fig. 3B). Addition of PHA to resting lymphocytes 2 h before labelling produced a prompt reduction in the wastage of 18S rRNA (Fig. 3C), the 28S : 18S labelling ratio falling to 1.75 (18S wastage = 18 per cent). Treatment with cycloheximide before addition of PHA prevented this increased 18S rRNA production (Fig. 3D). In the latter conditions, however, 45S RNA synthesis was stimulated. Clearly, therefore, the increase in 45S RNA synthesis which follows PHA treatment is not a consequence of reduced wastage of rRNA.

It is apparent from the effect of cycloheximide treatment that continuous protein synthesis is required for survival of new 18S rRNA (Fig. 3B,D)^{9,16,19}. This requirement was studied further to determine which step in 18S rRNA production may be sensitive to abolition of protein synthesis. The 45S rRNA precursor was labelled with [$\text{methyl-}^3\text{H}$]methionine during a 50 min pulse in resting lymphocytes. A 1 h chase was then performed, in the presence of a high dose of actinomycin D, in various conditions, and the 18S rRNA deficit calculated (Table 1).

The presence of cycloheximide during both the pulse and chase periods caused an increase in the normally high degree of 18S rRNA wastage in resting cells. If, however, protein synthesis was permitted to proceed normally during the pulse but was abolished by addition of cycloheximide during the chase, no increase in wastage occurred. It may be concluded that the availability of newly synthesized proteins during 45S RNA synthesis is sufficient to protect the 18S portion of about half of

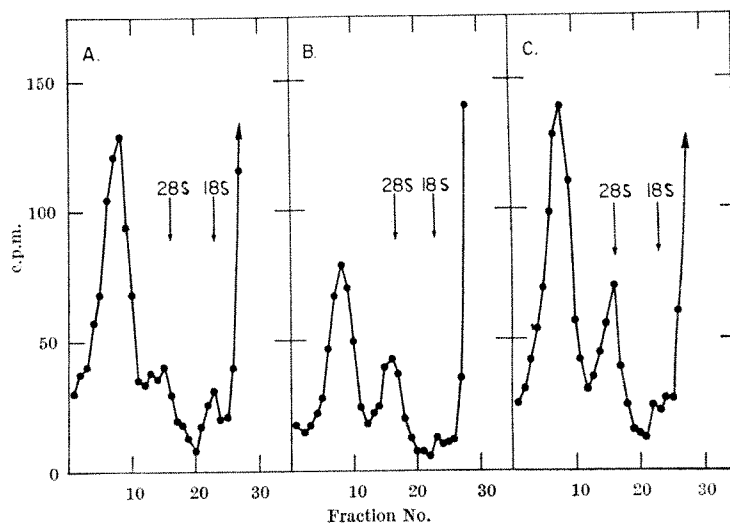


Fig. 2. Stimulation of 45S RNA synthesis by PHA in the absence of protein synthesis. Lymphocytes were incubated and labelled, after which RNA was extracted, as in Fig. 1. A, Resting cells; B, cycloheximide, 25 $\mu\text{g}/\text{ml}$, added 1 h before labelling; C, cycloheximide added 2.5 h before labelling; PHA added 1.5 h before labelling.

Table 1. WASTAGE OF 18S rRNA IN LYMPHOCYTES
50 minute pulse, 1 hour chase

Pulse	Additions to: Chase	Percentage 18S rRNA deficit
1. —	AMD	40
2. CHx	CHx+AMD	70
3. —	CHx+AMD	32
4. —	PHA+AMD	21
5. —	PHA+CHx+AMD	40

Results of a 50 min pulse, 1 h chase. Resting lymphocytes were incubated with [*methyl-³H*]methionine for 50 min (ref. 5). Medium was replaced with fresh medium containing excess unlabelled methionine and the additions shown. After 1 h RNA was extracted with phenol-sodium dodecyl sulphate at 40° C and sedimented through sucrose gradients¹². Distribution of radioactivity in 28S-32S and 18S peaks was determined as in Figs. 1-3 and the 28S : 18S labelling ratio calculated. On the basis of an expected 28S : 18S labelling ratio of 1.45 the percentage 18S rRNA deficit was calculated². Actinomycin D was used at a concentration of 10 µg/ml.

the new 45S molecules from subsequent degradation on cessation of protein synthesis. As little as 5 min is required in resting cells to abolish protein synthesis with the doses of cycloheximide used (unpublished). It is unlikely, therefore, that there was enough residual protein synthesis after addition of cycloheximide to explain the failure of the drug to increase 18S rRNA degradation when added only during the chase.

When PHA was added during the chase period, the normally high wastage of the 18S portion of previously synthesized 45S RNA was markedly reduced. A similar result has been observed, independently, by A. D. Rubin (personal communication). I observed this effect in the presence of a high dose of actinomycin, which has been shown to reduce lymphocyte RNA synthesis, almost immediately¹¹, to about 2 per cent of control levels (personal communication from R. Stern). But addition of cycloheximide together with PHA completely prevented this effect.

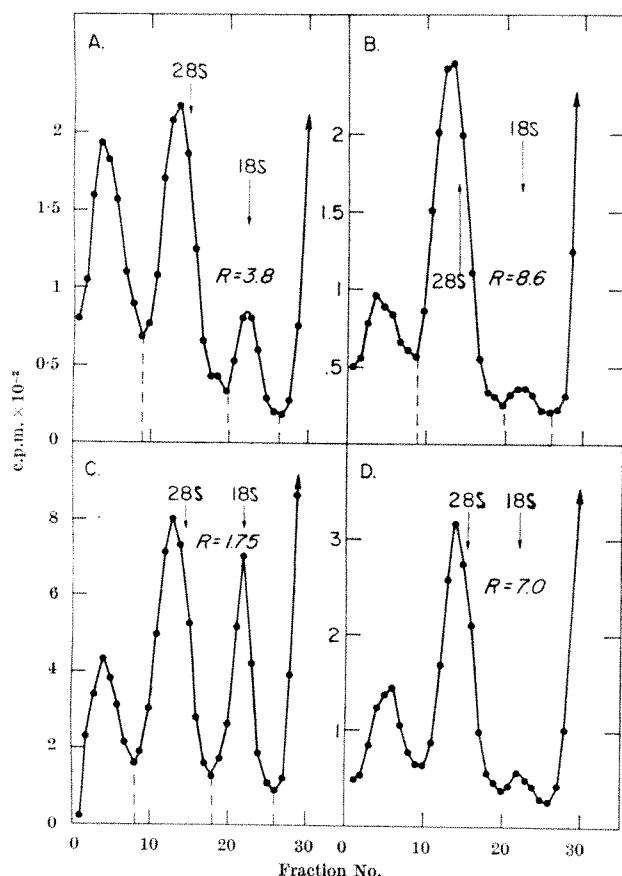


Fig. 3. Effect of PHA and cycloheximide on labelling of 18S rRNA. Lymphocytes were labelled with [*methyl-³H*]methionine for 2 h. Conditions otherwise as in Fig. 1. A, Resting cells; B, cycloheximide added 30 min before labelling; C, PHA added 2 h before labelling; D, cycloheximide added 2-5 h before labelling; PHA added 2 h before labelling. R designates ratio: (c.p.m. in 28S+32S)/(c.p.m. in 18S).

It seems that PHA can act very promptly to prevent degradation of a large portion of those 18S molecules synthesized in resting lymphocytes which would normally have been wasted. This action requires continuous protein synthesis, but does not require RNA synthesis. Thus both the normal survival of 18S rRNA, seen in resting cells, and the increased survival produced by PHA, are dependent on protein synthesis. Because, as noted, the rapid loss of new 18S rRNA molecules is accompanied by an equivalent but delayed loss of new 28S molecules, the availability of the required protein may set an upper limit on the rate of accumulation of new ribosomes. This may be an important growth regulating mechanism, for normal lymphocyte growth and division do not occur when ribosome production is blocked²⁰.

The action of PHA in reducing 18S rRNA wastage is independent of the PHA-induced rise in 45S RNA synthesis, for the former effect was observed in the presence of actinomycin. Further, the decrease of rRNA wastage requires intact protein synthesis, whereas increase of 45S RNA synthesis does not. Thus the two effects of PHA on rRNA metabolism seem to be independent and have different biochemical characteristics. Thus if a single primary PHA-induced event exists, it sets in motion more than one secondary series of events.

The findings have broader implications for the understanding of the control of gene action in animal cells. Thus 45S RNA is a primary gene product, albeit of a highly specialized nature. Although specialized processes have evolved to regulate its synthesis and utilization, it is also probable that certain basic steps are common to the handling of 45S RNA and the more elusive mRNA of the animal cell. The two steps examined here in regard to rRNA processing may have counterparts in the metabolism of mRNA. It is therefore likely that control at the transcriptional level exists for mRNA in animal cells, as it seems to exist for 45S RNA. More speculative, but highly intriguing, is the possibility that for mRNA, as for rRNA, a mechanism exists which determines the survival or degradation of newly synthesized mRNAs. This possibility has been raised by a number of workers²¹⁻²³. The large amount of heterogeneous, high molecular weight, labile RNA produced in the nuclei of all animal cells studied (reviewed in ref. 11), may include a pool of messages of which only those which are protected from immediate degradation will survive and be used. Whether such protection of mRNA is specific or non-specific, how it is mediated, indeed whether it exists at all, are questions for further study. The means by which new rRNA is protected from rapid degradation may serve as a starting point.

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Mutations affecting the Size of the Nucleolus in *Xenopus laevis*

by

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In *Xenopus laevis*, nucleolar mutants which form a partial nucleolus have been isolated. These provide a unique opportunity for the genetic analysis of the very reiterated ribosomal RNA genes.

THE nucleolus has always occupied an important place in the study of cell growth and protein synthesis, but its precise role was greatly clarified when it was shown to be the cellular organelle responsible for the synthesis of ribosomal RNA. The nucleolar organizer, which McClintock¹ defined as the chromosomal site necessary for the orderly organization of the nucleolus, is now known to be the location of the very reiterated ribosomal RNA genes^{2,3}.

The ribosomal RNA genes represent one of the few classes of reiterated genes for which a function is established, but it is not known if they are identical or how many are needed to permit the normal rate of ribosomal RNA synthesis and hence normal development. A detailed answer to these questions requires a particular class of mutants—one in which the normal number of ribosomal RNA genes has been reduced by deletion.

The ribosomal RNA genes are unique in that their rate of transcription and their distribution between the nucleolar organizers can be judged by cytological criteria⁴⁻⁷. More specifically, the relative abundance of ribosomal RNA genes in the two nucleolar organizers of a nucleus is reflected by the relative size of the two nucleoli⁷. We might therefore expect to be able to select the desired ribosomal gene mutants by the cytological analysis of nucleoli. *Xenopus* is particularly suitable for this type of work because large numbers of individuals can be cytologically analysed by examining a few cells from the tail tips of tadpoles which can then be reared to form a stock for genetic analysis. In this communication we outline the use of this approach to isolate partial nucleolar mutants and describe their origin, inheritance, cytological characteristics and development.

Characteristics of Partial Nucleolar Variants

The class of mutants in which we are interested would be expected to appear in heterozygous form as individuals with a large nucleolus (formed by a normal nucleolar organizer) and a partial nucleolus (formed by an organizer with a partial deletion of ribosomal RNA genes). We refer to this general class of nucleolar variants, having unequal sized nucleoli in most cells, as $+/p\ nu$. They are clearly distinguishable from wild type individuals which have two equal sized nucleoli ($+/+ \ nu$) in most cells (Fig. 1a and b). We can also recognize, within the $+/p\ nu$ variants, a category referred to as $+/p^1\ nu$. These are distinguished by the presence of extremely unequal sized nucleoli (Fig. 1c) and (as described later) by the lethality of the $0/p^1\ nu$ hemizygotes.

Table 1. NUCLEOLAR CONDITION OF PROGENY FROM SEVENTEEN CROSSES BETWEEN INDIVIDUALS HAVING TWO EQUAL NUCLEOLI

Nucleolar condition	Number of progeny*	Percentage
Two equal nucleoli ($+/+ \ nu$)	720	96.3
Two unequal nucleoli ($+/p\ nu$)	25	3.3
One nucleolus ($+/0 \ nu$)	3	0.4

* All of the progeny analysed were $+/+ \ nu$ in eight of the seventeen crosses and the $+/p\ nu$ embryos were found in the remaining nine crosses. Thirty-five embryos from each of the crosses were analysed.

To identify $p\ nu$ variants we have removed the tail tips of tadpoles at Nieuwkoop and Faber⁸ stage 32-42 for the examination of squash preparations by phase contrast microscopy. Nucleolar variants have been found among the progeny of frogs imported from Africa as well as among those of X-rayed frogs. Altogether, forty-one imported frogs have been analysed and five (12 per cent) produced progeny of which about half had slightly unequal nucleoli (Fig. 1b) when mated with $+/+ \ nu$ individuals. Thus these five frogs are probably heterozygous for an altered nucleolar organizer which forms only a partial nucleolus ($+/p\ nu$). This finding is consistent with the report that some individuals in natural populations of *Bufo marinus*⁷ and *Xenopus laevis*⁹ have unequal sized nucleoli or unequal nucleolar organizer constrictions¹⁰.

In the hope of increasing the frequency and severity of the $p\ nu$ variants, three male *Xenopus* were exposed to 1,200 rads of X-irradiation and mated within 3 days. Most of the progeny had a normal nucleolar constitution but a few variants were found with extremely unequal nucleoli. These $+/p^1\ nu$ variants (Fig. 1c) develop normally and are indistinguishable in viability from $+/+ \ nu$ animals.

The occasional origin of $p\ nu$ variants from crosses of two normal $+/+ \ nu$ parents has also been observed. The results of these crosses (Table 1) show that about half of the seventeen crosses between $+/+ \ nu$ frogs yielded some nucleolar variants, including some tadpoles with extremely unequal nucleoli, and three tadpoles with only one nucleolus per nucleus. These three tadpoles with one nucleolus per cell ($+/0 \ nu$, formerly known as 1- nu), have developed and metamorphosed normally, and represent the first reported cases of $+/0 \ nu$ frogs known to have arisen among the progeny of $+/+ \ nu$ parents. The 1- nu ($+/0 \ nu$) mutant of *Xenopus* originally described by Elsdale, Fischberg and Smith¹¹, was isolated from the normal laboratory stock. Similarly, two $+/0 \ nu$ individuals were found in a shipment of *Xenopus* from South Africa by Blackler⁹, who estimated that the frequency of these mutants in laboratory stocks taken from natural populations was about 1.5 per cent.

The presence of individuals with unequal nucleoli in our stock, and the discovery of $p\ nu$ variants among the progeny of X-rayed and normal parents, illustrate the susceptibility of the nucleolar organizer to alteration. Furthermore, the presence of $+/0 \ nu$ and $+/p\ nu$ variants among the progeny of $+/+ \ nu$ parents strongly suggests that the changes in the nucleolar organizer may take place during meiosis.

Inheritance of the $p\ nu$ Condition

To support the view that the partial nucleolar variants are the result of genetic rather than phenotypic variations, we have tested their inheritance through mitosis and meiosis. The inheritance of the $p\ nu$ condition through

mitosis was observed by comparing the nucleolar condition of cells in various tissues of the same individual. When this is done for tadpoles with slightly unequal nucleoli ($+p\ nu$) and those with very unequal nucleoli ($+p^i\ nu$), there is a limited variation among the cells of an individual; cells with equal nucleoli are rarely seen (15/100) in $+p\ nu$ tadpoles and never (0/500) in $+p^i\ nu$ tadpoles. Similarly, cells with obviously unequal nucleoli are very rarely seen (1/500) in $+p\ nu$ tadpoles.

The inheritance of the $p\ nu$ condition through meiosis has been followed in crosses between $+p\ nu$ and $+0\ nu$ frogs. In such crosses four types of progeny in equal numbers are expected if the $p\ nu$ condition segregates like a single Mendelian factor: $+/\ +\ nu$ (two equal nucleoli); $+p\ nu$ (unequal nucleoli); $+0\ nu$ (one nucleolus); and $p/0\ nu$ (one partial nucleolus). From such a cross, however, only three classes of progeny could be identified: about 25 per cent with two equal nucleoli, 25 per cent with two unequal nucleoli and 50 per cent with one nucleolus in each cell (Table 2). We assume that the nucleolar classes $+0\ nu$ and $p/0\ nu$ both appeared as tadpoles with a single large nucleolus in each nucleus. This result is expected in view of the well known ability of the nucleolar organizer genes to show dosage compensation, so that a constant amount of nucleolar material is organized irrespective of the number of organizers present^{7,12,13}. We have indeed observed that the size of the nucleolus formed by a $+ \nu$ nucleolar organizer is much greater when it shares a nucleus with a $p\ nu$ organizer than with another $+ \nu$ organizer.

Further evidence for the Mendelian inheritance of the $p\ nu$ condition comes from the analysis of twelve crosses between $+p^i\ nu$ frogs and $+0\ nu$ heterozygotes. The progeny of each cross include four classes of nucleolar conditions (Table 2). $p^i/0\ nu$ embryos have a single nucleolus in most cells, but can be distinguished from $+0\ nu$ tadpoles by the presence of some cells with 1-4 very small nucleolus-like bodies (Fig. 1d). These nucleolus-like bodies are variable in size and are similar to those found in the anucleolate mutants of *Xenopus*¹¹, *Chironomus*¹³ and *Zea mays*¹. In contrast to the anucleolate mutants, however, the extra nucleolar bodies of $p^i/0\ nu$ mutants are only found in small numbers, usually one or two per nucleus. In fixed preparations stained with methyl green and thionine¹⁴, the nucleoli and nucleolus-like bodies stain metachromatically as expected for structures containing RNA.

Table 2. NUCLEOLAR CONDITION OF PROGENY FROM CROSSES BETWEEN $+0\ nu$ AND $+p\ nu$ MUTANTS

Nucleolar condition of progeny	Cross and number of progeny	
	$+0\ nu \times +p\ nu$	$+0\ nu \times +p^i\ nu$
Two equal nucleoli ($+/\ +\ nu$)	53	101
Two unequal nucleoli ($+p\ nu$ or $+p^i\ nu$)	46	96
One nucleolus ($+0\ nu$)	101	94
One nucleolus ($p/0\ nu$ or $p^i/0\ nu$ *)		84*

* Distinguished from $+0\ nu$ by the presence of extra nucleolus-like bodies in some cells.

It is of interest to note that the $p^i\ nu$ organizer forms a single large nucleolus in many cells of the $p^i/0\ nu$ embryos. These nucleoli may not be normal, however, for they appear to have an abnormally high density when compared with normal nucleoli under the phase contrast microscope. Similar observations have been reported for the anucleolate mutant of *Xenopus*¹⁵, and it has been shown that the pseudonucleolus found in these mutants has an abnormal ultrastructure¹⁶.

We conclude from these tests that the $p\ nu$ condition is a mutation which is inherited as if it were a single Mendelian factor.

Development of $p\ nu$ Mutants

Analysis of the developmental effects of the partial nucleolar mutations has been greatly facilitated by

combining a $p\ nu$ or $p^i\ nu$ chromosome with a homologous chromosome totally lacking a nucleolar organizer. This is accomplished by crossing a $+p\ nu$ or $+p^i\ nu$ heterozygote with a $+0\ nu$ frog. The $p^i/0\ nu$ embryos, with a single large nucleolus in each cell, develop and metamorphose normally. In contrast, $p/0\ nu$ embryos, which contain extra nucleolus-like bodies in addition to a large nucleolus in some of their cells, die as tadpoles before starting to feed. Phenotypically the $p^i/0\ nu$ embryos are normal during the early stages of development but, after hatching, they become microcephalic, develop oedemata in the regions of the eyes, heart and abdomen, and at later stages their bodies become dorso-ventrally bent. They stop further development at stage 42 and die 9-12 days after fertilization when their normal sibs have reached stage 47 or 48.

The developmental abnormalities of the $p^i/0\ nu$ mutants are very similar to those of anucleolate mutants of *Xenopus*¹¹, but $p^i/0\ nu$ embryos live a few days longer. In order to rule out maternal effects as an explanation of longer survival, the relative length of survival permitted by these two lethal conditions was determined by mating the same $+0\ nu$ female successively with a $+0\ nu$ male and a $+p^i\ nu$ male. Progeny carrying only the $p^i\ nu$ organizer lived for 3 days longer than the anucleolate progeny ($0/0\ nu$).

Usefulness of the $p\ nu$ Mutants of *Xenopus*

The $p\ nu$ variants described in this report, and called mutants on account of the Mendelian inheritance of the $p\ nu$ conditions through meiosis, have been shown to have the following characteristics: (1) they are characterized cytologically by the presence of nucleoli of unequal size in most cells; (2) they occur quite frequently in natural populations and the $p\ nu$ mutation probably arises during meiosis; (3) such mutants fall into two classes, defined according to the viability or inviability of individuals whose only nucleolar material is formed by one $p\ nu$ organizer ($p/0\ nu$ and $p^i/0\ nu$ mutants).

Partial nucleolar mutants of the kind described here will be of particular value for the genetic analysis of ribosomal RNA genes whenever the $p\ nu$ nucleolar organizers are found to have less than the normal number of these genes. An analysis of the ribosomal RNA synthesized by different $p\ nu$ mutants may make it possible to determine if the highly reiterated ribosomal RNA genes are heterogeneous. If they are, then complementation studies using different $p\ nu$ mutants would be invaluable in any attempt to investigate the nature of these differences. If ribosomal RNA genes consist of repeated identical sets, then $p\ nu$ mutants would make it possible to determine the minimum number of such sets necessary for normal cell growth and function. Results to be presented elsewhere show that the $p^i/0\ nu$ mutant described here synthesizes ribosomal RNA at a reduced rate¹⁷ and has considerably less than the haploid number of ribosomal RNA genes¹⁸.

Mutants involving partial deletions of ribosomal RNA genes have been collected in *Bufo marinus*⁷ and *Drosophila melanogaster*¹⁹. In spite of the great convenience of *Drosophila* for breeding experiments, we believe that $p\ nu$ mutants of *Xenopus* offer several important advantages for analysis of ribosomal RNA genes.

(1) The use of phase contrast microscopy to examine the nucleoli of a small number of cells taken from living embryos permits easy identification and collection of heterozygous $p\ nu$ mutants.

(2) *Xenopus* embryos are sufficiently large to permit the biochemical analysis of a single tadpole¹⁸. Unless this can be done, it is necessary to combine several individuals for a biochemical analysis. This could be a substantial disadvantage because siblings are not certain to have exactly the same genotype in view of the changes which can occur in the ribosomal RNA genes at meiosis.

(3) The maternal ribosome supply of *Xenopus* embryos makes it possible to study ribosomal RNA synthesis in lethal mutants having very small numbers of ribosomal RNA genes¹⁸.

(4) The use of *Xenopus* permits the vegetative propagation of mutant embryos by nuclear transplantation²⁰. The capacity of mutant cells for growth, as opposed to differentiation, can be tested by culturing cells from single tadpoles²¹.

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Spotted Hyaena: Crusher, Gnawer, Digester and Collector of Bones

by

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Studies in East Africa confirm that spotted hyaenas sometimes accumulate bones in their lairs. Four types of bone damage caused by the hyaenas are defined.

THE anthropologist, studying excavated bone remains, frequently faces the problem of distinguishing hominid-worked specimens (the earliest supposed examples being from deposits of Miocene age¹), from those damaged by other processes. Because the least robust parts of all bones are likely to be destroyed more readily than stronger parts, whatever the destructive process, bone fragments of similar appearance can result from different causes and special care must be taken to differentiate between them.

Brain, for example, has pointed out² that, in South Africa, desert weathering of bone fragments, followed by abrasion in sand disturbed by feet of animals and people, can lead to the formation of pseudo-tools; and that differential durability of goat bones, discarded by Hottentot tribesmen and gnawed by their dogs, can result in a disproportionate representation of the various skeletal parts³. Washburn has observed⁴ that eating by carnivores is normally a highly selective process; and, in Europe, palaeontologists have repeatedly remarked on the consistent form of bone remains supposed to have been left in caves by Pleistocene spotted hyaenas, compact bone surviving more readily than cancellous bone⁵⁻⁸. Dart, following his studies of australopithecine sites in South Africa, has questioned⁹ whether the European Pleistocene hyaena was in fact the traditionally accepted accumulator of bones, suggesting instead that the fractured hyaena remains found at such sites as Kirkdale Cave in Yorkshire should be attributed to early man. Thenius⁷ has commented on the close similarity between the fracturing of bones attributed by Dart to the osteodontokeratic culture of *Australopithecus prometheus* and that of bones fed by Zapfe¹⁰ to a spotted hyaena in the Schönbrunn Zoo, Vienna (see also Wolberg¹¹). Porcupines^{6,12,13}; leopards^{12,14}; tigers, lions, wolves and coyotes¹⁵; are further animals known to damage bones.

The nature of hyaena damage and the process of accumulation of hyaena damaged bones is especially complex and presents a constant potential source of

confusion for those studying remains from hominid sites. I propose to place on record here some observations on the habits of wild spotted hyaenas noted during field work in East Africa during 1960 and 1967.

Feeding and Bone Accumulating Habits of Spotted Hyaenas

The feeding and denning habits of spotted hyaenas have been previously studied by a number of workers. Hughes found¹⁶ that in South Africa, the spotted hyaena habitually feeds where it finds its food. He concluded that it carries away, if anything, only easily digested fragments suitable for feeding its young. Kruuk has shown¹⁷ that hyaenas are not only the proverbial scavengers but that, in the Ngorongoro Crater, Tanzania, they also chase and kill wildebeest and zebra. These are normally consumed in the open. Inspection, after about



Fig. 1. Details of carcass of a wildebeest, killed and partly eaten by spotted hyaenas in the Ngorongoro Crater, Tanzania. Photographed about 20 min after death. Note splintering of the tibia.

twenty minutes of a carcass of a wildebeest, killed there by spotted hyaenas under the observation of Kruuk and myself, showed that the ventral part of the rib cage had already been broken away, all the soft internal parts had been consumed, a tibia had been broken across and part was missing (Fig. 1).

Matthews, from his observations in East Africa¹⁸, recorded that spotted hyaenas always use a burrow or den of some sort as a nursery for their young. He described two types of lair: in natural cavities between granite boulders in small hills known locally as kopjes; and in burrows excavated in soft sediments. Hughes described¹⁹ similar lairs of both types in the Kruger National Park, South Africa, and also made an extensive search^{13, 16, 19, 20}, both in the field and through the press, for hyaena lairs which might be found to contain accumulations of bones. Nevertheless, the inspection of various lairs and the complete excavation of two of them did not reveal any accumulations of bones or of hyaena droppings. He concluded that the hyaena probably frequents caves, earths or rockshelters only when it has its young; that it does not habitually collect great quantities of bones in or near its lair; and that it does not foul the place where it sleeps or has its young.

During 1967, I inspected about twenty East African caves, the most important being on Mt Elgon (in Miocene agglomerate) and on Mt Suswa, Kenya (lava caves), with the object of looking for bone deposits. These caves differed from the hyaena lairs mentioned earlier in being large and easily explored throughout most of their distance. Although it was clear that many of these caves were frequented by hyaenas, especially in the daylight zone near the entrance, because splintered bone fragments and occasional droppings (including some groups of droppings in the dark zone) were present, nowhere could the bones be described as an accumulation. But investigation of a number of lairs of spotted hyaenas, similar to those described by Matthews and Hughes, produced strikingly different results.

In the Queen Elizabeth Park, Uganda, two burrows in alluvial sediment (both too constricted for human access) were, with permission from the Director of National Parks, completely excavated. The first was of



Fig. 2. Bone remains obtained by excavation from the entrance tunnels of a spotted hyaena lair in the Queen Elizabeth National Park, Uganda. They include skulls of kob, baby hippopotamus and warthog; antelope jaws and (centre) a humerus of a baby elephant.



Fig. 3. Interior of a spotted hyaena lair, near Kajiado, Kenya. Note human cranium and remains of domestic animals.

simple plan with a single terminal chamber, about three feet below ground level, opening to the surface by a trifurcating tunnel. A few fragments of bones of antelopes and of a juvenile hippopotamus were found in the access tunnel. The terminal chamber was empty except for the complete skeleton of a baby spotted hyaena and one dropping. The second lair was more complicated, having a central chamber (which was empty) and a series of tunnels leading to at least ten openings situated around it. The tunnels contained numerous bone remains, including skulls of baby hippopotamus, warthog and kob, bones of buffalo, a humerus of a baby elephant (Fig. 2) and a fragment of a skull of a baby hyaena. Many of these remains were splintered and gnawed.

A spotted hyaena lair near the side of the lake in the Ngorongoro Crater was found to be sufficiently large to permit human access for a short distance inside some of its entrances, although all tunnels soon became too small for further progress. A substantial quantity of bones, principally of zebra and wildebeest, but also including an almost complete skull of an adult hyaena, was collected with a pole from inside the lair. An extensive scatter of bone fragments, many splintered, was also found outside. There could be no doubt that hyaenas were the principal occupants of the lair and were responsible for most of the bones found there. I was rewarded with a view of the head and shoulders of a three-quarter grown hyaena inside a tunnel before the animal nervously backed away. Another partly grown hyaena was seen carrying a bone into another hole in the crater floor.

I also inspected two further hyaena lairs, near Kajiado, Kenya. Both were natural horizontal cracks in lava which apparently extended some distance into the rock, but were too low for human exploration beyond about 15 feet. Both tunnels contained substantial quantities of bones (including those of domestic donkey, cow and dog), one of them for a distance of at least 25 feet. In this lair were also ostrich bones, part of an ostrich egg, a human jaw, three human crania (Fig. 3) and a few other human remains. A great quantity of bone fragments were also strewn about outside. Both lairs could reliably be attributed to spotted hyaenas. Both were close to Masai villages, whence the remains of the domestic animals doubtless originated. Three local Masai villagers independently expressed the opinion that human skulls had been carried to the lair from Kajiado Hospital cemetery, about two and a half miles away, by hyaenas. An inspection of the cemetery showed that many of the graves had indeed been plundered by hyaenas and that there were innumerable splintered human bone fragments scattered around them.



Fig. 4. Gnaw marks caused by the milk teeth of spotted hyaenas on a humerus and metatarsal of wildebeest, from inside a lair in the Ngorongoro Crater (scale in cm) (photo by courtesy of BM(NH)).

From these observations it seems that although adult East African spotted hyaenas normally feed in the open, young animals nevertheless commonly carry bones into their lairs. These bones may have originated a considerable distance away. The bones found in the lairs are predominantly remains of animals killed or scavenged locally (principally herbivores), generally less than 5 per cent being of spotted hyaenas. Hyaena droppings are usually absent. Lairs containing young hyaenas are characteristically too small for human ingress and should therefore generally be distinguishable from hominid dwelling caves on grounds of size.

My present investigations suggest that there may be regional differences in the behaviour of spotted hyaenas, those in East Africa having a greater tendency to carry bones to their lairs than those of South Africa although the reason for this is probably merely the proximity of the food supply. The British Pleistocene hyaena (sometimes represented by deposits composed of 90 per cent hyaena remains, contained in tunnels too small to be suitable for human habitation) may perhaps have hibernated in caves, like the great cave bear, *Ursus spelaeus*. No comparable accumulations of hyaena bones, recent or fossil, have yet been recorded from any African site.

Types of Bone Damage caused by Spotted Hyaenas

From the above studies, four types of bone damage caused by spotted hyaenas may be defined:

Type 1: splintering of bones by adult animals. The adult spotted hyaena commonly splinters bones, with the aid of its powerful premolars. The structural specialization whereby it can seize and crush its prey has been described by Buckland-Wright²¹. A typical splintered bone is shown in Fig. 1. A study of bone fragments from the sites described above shows that there is a regular pattern of those parts of bones which survive and those which are eaten; the robust parts surviving, whereas cancellous bone is more commonly destroyed. This situation is especially well demonstrated by human remains collected around the graves at Kajiado Hospital cemetery. These consisted predominantly of the shafts of tibiae, femora and humeri, all with their ends missing, some skull remains and very little else. It is significant that the shafts of human limb

bones are composed of compact bone, the ends mostly of cancellous bone. Similar bone damage has been observed by Brain, who experimentally fed baboon remains to cheetahs¹². These animals ate the whole vertebral column, hands and feet, and also caused damage to the ends of the limb bones.

Similarly, the distal ends of artiodactyl humeri tend to survive, whereas the proximal ends are often missing; and bovid and equid metapodials often survive complete. The ascending ramus and ventral margins of herbivore jaws are frequently bitten away and the upper part of a human skull is more likely to survive than the facial region. Similar patterns of bone breakage can be found for all skeletal parts of all animals.

Type 2: gnawing of bones by juvenile animals. Juvenile hyaenas which still have their milk dentition apparently cannot splinter bones, as the adults do, but gnaw them, leaving striations at right angles to the elongation of bones (Fig. 4). Examples of such gnawing were found on many of the bones extracted from the lairs in the Queen Elizabeth Park and the Ngorongoro Crater, described earlier. Because most such bones had originally been torn from the carcass by adult hyaenas, before being brought to the lair, both type 1 and type 2 bone damage were frequently found on the same specimen. Close inspection of such gnawing shows the striations to have been formed by tearing and that they are seldom straight for more than a short distance. The striations are probably indistinguishable from the gnawings of other medium-sized carnivores (similar gnawing by coyotes has been illustrated by Miller¹⁵), but they are quite distinct from the chiselled tooth marks of porcupines and from marks made by knives. Continued gnawing by juvenile hyaenas can sometimes lead to the formation of holes in bones. In this way a captive juvenile spotted hyaena, owned by M. Croydon, of Nairobi, created a "window" in the shaft of an antelope metatarsal, close to its distal end.

Type 3: scooping out of cancellous bone. Zapfe, from his studies at the Schönbrunn Zoo, observed¹⁰ that the scooping out of spongy bone is characteristic of carnivore feeding. This seems to be a feature predominantly of bones too large for hyaenas to break across. It is well shown in a bovid femur gnawed by Croydon's captive juvenile hyaena, where the walls of the greater trochanter were left standing as a thin circle of bone around a scooped out central pit.

Type 4: damage by partial digestion. In the course of feeding, adult hyaenas frequently swallow substantial fragments of bones. After feeding they subsequently regurgitate everything indigestible. Numerous piles of regurgitated food material were found around the

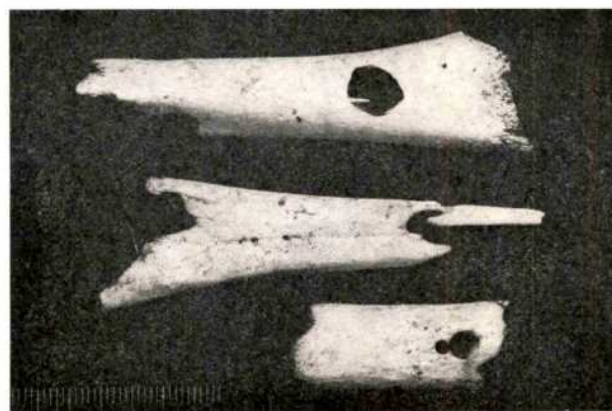


Fig. 5. Bones of juvenile wildebeest from the Ngorongoro Crater, partly digested and subsequently regurgitated (in the open) by spotted hyaenas. Note circular holes and bone fragment lodged inside the uppermost specimen (scale in mm) (photo by courtesy of BM(NH)).

entrances of the lair in the Ngorongoro Crater, described earlier. These consisted not only of partially digested bone fragments and teeth, mostly of juvenile wildebeest (Fig. 5) but also hoofs and great quantities of hair, some of it compacted tightly inside the bone fragments. Distinctive features are: scalloping of the bone surface; knife sharp edges between two eroded faces; and even circular holes. The remains have the appearance of the hyaena's gastric juices having been circulated around them under pressure, since not only were the bones packed with masses of hair, but some of the hairs were threaded through the minute channels in the bone and small pieces of bone were even wedged inside the larger fragments. Although these regurgitated tooth and bone remains, especially bone fragments with circular holes in them, could be mistaken for human artefacts (Kitching, for example, has pointed out²² that the object from Pin Hole Cave, Derbyshire, previously thought to be a "bull-roarer" did, in fact, result from erosion by gastric juices, probably of a hyaena) they are generally distinctive and should be easily recognized by those studying damaged bone remains.

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Mass Fractionation and Isotope Anomalies in Neon and Xenon

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The neon and xenon isotope anomalies observed in meteorites can be interpreted in terms of a mass fractionation, which is common for both elements. It is not necessary to assume the existence of large excesses of fission-type xenon in carbonaceous and gas-rich chondrites.

THE role of mass fractionation in neon and xenon isotope anomalies has never been clearly understood. Reynolds¹ discussed this problem and reported it as "unexplained isotope anomalies in neon and xenon". Marti² has recently suggested that terrestrial atmospheric and solar-type xenon may be related to each other by a strong mass fractionation process, while trapped chondritic xenon might be related to solar-type xenon by superimposing fission-xenon components with the required relative mass yields on the latter. According to Anders and Heymann³, the fission component found in carbonaceous and gas-rich chondrites was not derived from an actinide element ($Z = 89$ to 103), or from a transition metal between $Z = 104$ and 111, but from a more volatile progenitor ($Z = 112$ to 119).

The isotopic compositions of neon in various chondrites seem to be quite complex. According to Pepin⁴, a number of different components of neon occur in nature, and there is no sign that diffusive fractionation is responsible for any of the isotopic variations. Manuel⁵, on the other hand, reported that the isotopic composition of neon observed in the gas-rich chondrite Fayetteville could be produced from a mixture of primordial neon, cosmogenic neon and highly mass-fractionated primordial neon.

We re-examine here the published xenon isotope ratio data from the stepwise heating experiments on the Fayetteville⁶, Renazzo⁶, Mokoia and Chainpur⁷ chondrites. These experimental data are plotted in Fig. 1 together

with the xenon isotope ratios in the atmosphere⁸, in average carbonaceous chondrites⁹, and in the 800° fraction of lunar fines¹⁰.

The solid lines I, II, III and IV in Fig. 1 correspond to the equations

$$y = 5.15x - 3.03 \quad (1)$$

$$y = 5.07x - 3.58 \quad (2)$$

$$y = 1.17x - 0.92 \quad (3)$$

$$y = 0.735x - 0.649 \quad (4)$$

where $x = (^{134}\text{Xe}/^{136}\text{Xe})_{\text{met}}$, $y = (i\text{Xe}/^{136}\text{Xe})_{\text{met}}$ and $i = 132, 131, 130$ and 128.

Equations (1)–(4) can be derived from a general equation

$$f = \left(\frac{(i\text{Xe}/^{136}\text{Xe})_{\text{met}}}{(i\text{Xe}/^{136}\text{Xe})_{\text{atm}}} - 1 \right) \cdot \frac{136}{(136 - i)} \quad (5)$$

where f may be defined as a mass fractionation factor for the xenon isotopes.

The dashed lines a , b , c and d in Fig. 1 are parallel to the straight lines I, II, III and IV, respectively, and pass through most of the experimental points. By extrapolating the straight lines a , b and c to the left, we can find the values of $^{134}\text{Xe}/^{136}\text{Xe}$, $^{132}\text{Xe}/^{136}\text{Xe}$ and $^{131}\text{Xe}/^{136}\text{Xe}$, when the $^{130}\text{Xe}/^{136}\text{Xe}$ ratio become zero. From these values,

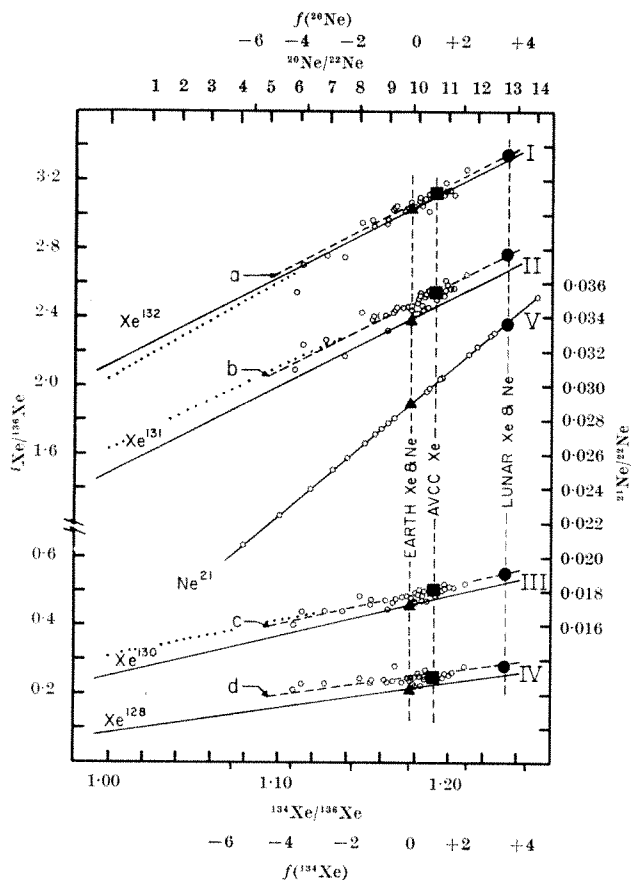


Fig. 1. A comparison of atmospheric⁶, lunar¹² and AVCC⁹ xenon with the xenon released from the Fayetteville⁵, Renazzo⁸, Mokoia and Chainpur⁷ chondrites. The solid lines through atmospheric xenon show the isotopic composition of mass fractionated xenon, the dashed lines show the isotopic composition of mass fractionated AVCC xenon, and the dotted lines show the isotopic composition of xenon produced by mixing AVCC xenon with the "fission" xenon defined by Pepin⁴. The xenon coordinates are labelled on the lower abscissa and on the left ordinate. The isotopic composition of atmospheric¹³ and lunar¹² neon is also compared with the neon released from Fayetteville⁵ and Renazzo⁸. The neon coordinates are defined on the upper abscissa and on the right ordinate.

we can secure the $^{131}\text{Xe} : ^{132}\text{Xe} : ^{134}\text{Xe} : ^{136}\text{Xe}$ ratio, which represent the mass-yield distribution of the "unknown" fission component discovered by previous investigators.

According to Pepin⁴, this ratio is

$$^{131}\text{Xe} = 0.25 \pm 0.13, \quad ^{132}\text{Xe} = 0.48 \pm 0.08, \quad ^{134}\text{Xe} = 1.000 \quad \text{and} \\ ^{136}\text{Xe} = 1.43 \pm 0.06$$

The dotted lines in Fig. 1 connect AVCC xenon⁹ to the above mass-yield distribution. The dotted "fission" lines are distinct from the dashed "mass fractionation" lines primarily in the regions to the left of the experimental data points, except for the $^{132}\text{Xe}/^{136}\text{Xe}$ ratios, where several of the data points lie below both lines. This behaviour is to be expected if these chondrites contain xenon isotopes from the spontaneous fission of 82 m.y. ^{244}Pu . The amounts of fissionogenic xenon indicated by the deviations of the $^{132}\text{Xe}/^{136}\text{Xe}$ ratios below the mass fractionation line are in all cases less than the amounts of xenon produced by ^{244}Pu in Pasamonte¹¹.

Are the xenon isotope ratio data caused either by mass fractionation or by fission of an unknown element? Fission of an unknown element is certainly an attractive explanation, because the unknown element may be one of the superheavy elements ($Z=112$ to 119), as suggested by Anders and Heymann³. But, the fact that the mass-yield distribution deduced from the xenon isotope data is so close to that deduced from the mass fractionation

theory is most disquieting. Moreover, this explanation does not help us to understand the neon isotope anomalies, which are just as spectacular as the xenon isotope anomalies. Mass fractionation, on the other hand, is a promising explanation, because it helps in understanding the phenomena of isotope anomalies in rare gases in general.

Fig. 1 compares the neon data obtained by Manuel⁵ and by Reynolds and Turner⁶ with lunar¹² and atmospheric¹³ neon. The neon isotope ratios for meteorites have been corrected for cosmogenic neon by the method described by Manuel⁵, but no corrections were made on the lunar and atmospheric neon ratios. The experimental data fit line V in Fig. 1 which is defined by

$$y = 1.481 \times 10^{-3}x + 0.01452 \quad (6)$$

where $x = (^{20}\text{Ne}/^{22}\text{Ne})_{\text{met}}$ and $y = (^{21}\text{Ne}/^{22}\text{Ne})_{\text{met}}$.

By writing the equation (5) in a more general form

$$f = \left\{ \frac{(X^i/X^j)_{\text{met}}}{(X^i/X^j)_{\text{atm}}} - 1 \right\} \cdot \frac{j}{(j-i)} \quad (7)$$

where X may be any one of the rare gas elements and i and j are the mass numbers, we can derive equation (6) from equation (7) and also calculate the values of f for the neon isotopes. The f values for neon and xenon are also indicated on the abscissae of Fig. 1. Similar relationships between $^{20}\text{Ne}/^{22}\text{Ne}$ and $^{36}\text{Ar}/^{38}\text{Ar}$ and also between $^{20}\text{Ne}/^{22}\text{Ne}$ and $^{20}\text{Ne}/^{36}\text{Ar}$ in some chondrites were found several years ago by Signer and Suess¹⁵. Kuroda¹⁶ attempted unsuccessfully to calculate the effect of mass-fractionation on atmospheric xenon using the equation given by Suess¹⁷

$$-\log \frac{N_{\text{ter}}}{N_{\text{sol}}} = 10 \cdot 0.045 M/m_1 + 7.1 \quad (8)$$

where N_{ter} and N_{sol} are the terrestrial and the cosmic abundances of the rare gases with the mass M/m_1 , respec-

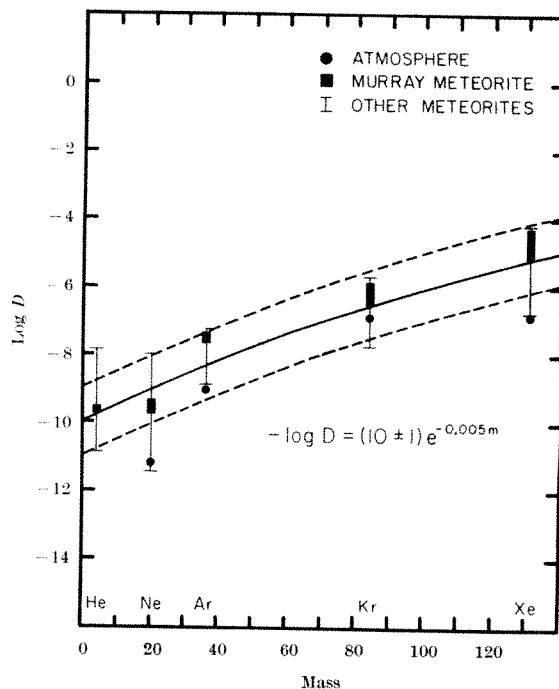


Fig. 2. A comparison of the abundance pattern of noble gases predicted by equation (9) with the abundances of noble gases in the atmosphere, the Murray carbonaceous chondrite, and sixteen other meteorites containing primordial noble gases. The data points are from Signer and Suess¹⁵.

tively. Equation (8) gives $f = +0.325$ for cosmic xenon and $f = 13.62$ for cosmic neon, calculated for the $^{134}\text{Xe}/^{136}\text{Xe}$ and $^{20}\text{Ne}/^{22}\text{Ne}$ ratios, respectively. Obviously, the Suess equation does not predict the magnitudes of the effect of mass fractionation that are observed in atmospheric noble gases.

In deriving equation (8), Suess assumed that essentially all of the Earth's noble gases reside in the atmosphere where there is no evidence of any significant depletion of Kr from Xe, although both gases are depleted to about 10^{-7} of their cosmic abundances. The term 7.1 in equation (8) represents the loss of gases by a non-fractionating process to $10^{-7.1}$ of their cosmic abundance. In chondrites, however, the abundance pattern of noble gases shows a separation of krypton from xenon by about a factor of 10, and recent work on terrestrial samples¹⁸ indicates that fractionation patterns for noble gases in chondrites and in the Earth are very similar if the preferential retention of xenon in certain terrestrial rocks and minerals is taken into account.

Because the degree of mass fractionation observed in meteoritic noble gases varies with the extraction temperature, it is apparent that the isotope fractionation of a gas observed in different temperature fractions may not be related to the extent to which the total abundance of the gas is depleted below its cosmic abundance. Nevertheless, the approximately equal range of f values for neon and xenon shown in Fig. 1 can be obtained by removing the term 7.1 from the Suess equation and adjusting the coeffi-

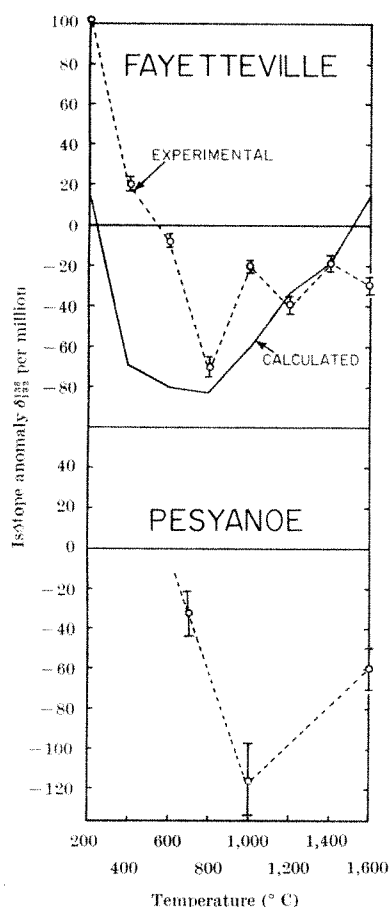


Fig. 3. Temperature release experiments on the gas-rich chondrites Fayetteville and Pesyanoe. Experimental data (---) were obtained by Manuel⁸ and Marti⁹, respectively. The calculated curve for Fayetteville (—) was obtained from the neon data by Manuel, assuming that the mass fractionation process is common for neon and xenon. Small contributions from the neutron capture and cosmic-ray spallation reactions at mass number 132 are disregarded in the calculations.

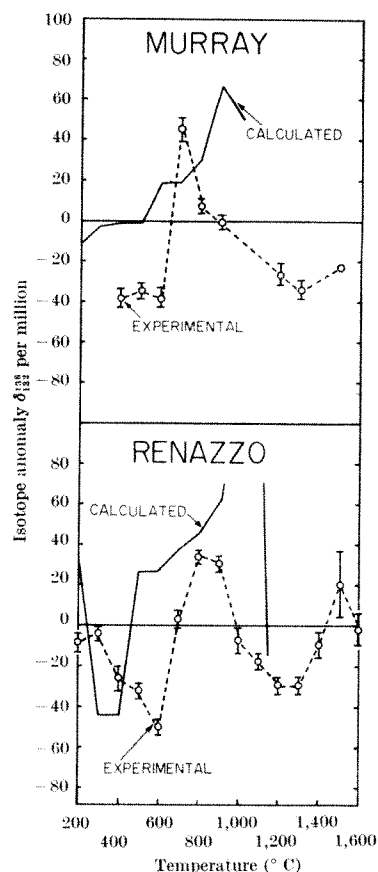


Fig. 4. Temperature release experiments on the carbonaceous chondrites Murray and Renazzo. Experimental data (---) were reported by Reynolds¹⁴ and Reynolds and Turner⁶, respectively. The calculated curve for Murray (—) was obtained from the neon data reported by Pepin⁴. The neon data for Renazzo were reported by Reynolds and Turner⁶. Because of the presence of cosmic ray-produced neon, the neon data are unreliable above $1,000^{\circ}\text{C}$. Small contributions from the neutron capture and cosmic-ray spallation reactions at mass number 132 are again disregarded in the calculations.

cient to fit the abundances of primordial noble gases

$$-\log D = (10 \pm 1)e^{-0.005m} \quad (9)$$

where D is the ratio of the terrestrial or meteoritic to the cosmic abundances of a rare gas isotope with mass number m . Fig 2 compares the fractionation pattern of noble gases predicted by equation (9) with the abundances of primordial noble gases in the atmosphere and the Murray chondrite. The range of noble gas abundances for sixteen other meteorites is also shown.

Equation (9) predicts $f = +7.6$ for cosmic xenon and $f = +2.5$ for cosmic neon, whereas the Suess equation predicted much smaller f values for xenon (0.325) than neon (13.62). From Fig. 1, it seems that approximately equivalent f values will relate atmospheric neon and xenon to solar-type neon ($f = +3.24$) and xenon ($f = +3.58$), and that there is an approximately equal range of f values observed in the temperature release patterns for neon and xenon from meteorites. To test for a common fractionation of neon and xenon isotopes in the temperature release patterns from carbonaceous and gas-rich meteorites, we calculate f values from the neon data and use these values to calculate the δ_{132}^{136} values for xenon, defined as

$$\delta_{132}^{136} = 100 \left\{ \frac{(^{136}\text{Xe}/^{132}\text{Xe})_{\text{met}}}{(^{136}\text{Xe}/^{132}\text{Xe})_{\text{atm}}} - 1 \right\} \quad (10)$$

Figs. 3 and 4 compare the calculated and experimental values from the stepwise heating experiments on a number of meteorites.

Although the agreement is not perfect, the theory does predict the general behaviour of the xenon temperature release patterns. In the case of Fayetteville, there is a minimum at 1,000° C. The release pattern for Pesyanoe seems to be similar to that for Fayetteville. A peak is observed just below 1,000° C in the cases of Murray and Renazzo. A sharp minimum observed at Renazzo 600° C fraction corresponds to a minimum in the calculated curve (—) at 300°–400° C fractions.

The crucial test of the proposed new interpretation for the rare gas isotope anomalies in terms of mass fractionation should come from the studies on the isotope anomalies in krypton. The krypton data are scarce and we are now in the process of securing reliable krypton data in our laboratories.

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Radio Pulses from Extensive Air Showers

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A relationship has been obtained between radio pulse amplitudes and the shower parameters given by particle detectors.

RADIO pulses associated with extensive air showers have been detected by many investigators during the past five years (for references, see ref. 1). Quantitative information is still sparse, however. An average lateral distribution function for the radiation at 30 MHz has been given by Vernov *et al.*² but only a small number of showers were available, and fluctuations between one shower and another were very large. Similar trouble with fluctuations has been experienced at Haverah Park³, and by the Ann Arbor group at Chacaltaya⁴. For this reason, most results have been expressed statistically, by the proportion of showers in different categories that give detectable radio pulses (see, for instance, ref. 4). At Haverah Park we have now succeeded in obtaining what is essentially a 1:1 relationship between radio pulses amplitude and the shower parameters given by the particle detectors. Some scatter remains (~a factor of two in radio pulse amplitude) but this can reasonably be attributed to the known uncertainties in the particle parameters.

The improvement has come about chiefly by the adoption of 55 MHz as the principal measuring frequency. For the showers detected at Haverah Park, with primary energies $E_p \sim 10^{17}$ to 10^{18} eV, the radio pulses are only a few times larger than the r.m.s. background noise. Most of the noise power is in a quasi-thermal component, but at the frequencies we have used previously (32 MHz and 44 MHz) there is a second component in the background, consisting of bandwidth-limited pulses typically five times larger than the thermal component and occurring at intervals of a few μ s. When such a pulse occurs in time coincidence with the arrival of an air shower, there is no way of distinguishing it from a genuine radio pulse originating in the air shower. The large background pulses are numerous at 32 MHz, less frequent at 44 MHz and at

55 MHz they are not observed at all. From the way their number varies between summer and winter⁵ we speculate that they may be dependent on seasonal changes in the ionosphere, analogous perhaps to the anomalous effects at lower frequencies discussed by Appleton and Piggott⁶ and by Minnis⁷. At 32 MHz, measurements on air shower radio pulses are practicable only during the winter, and even then some 20 per cent of the pulses ascribed to the air showers are probably spurious¹. At 55 MHz we are able to identify an air shower pulse unequivocally if it has an amplitude appreciably larger than the thermal background.

All measurements on the radio pulses have been made during the night (0–0800 h) because of the additional man-made interference experienced during the day from sound and television broadcasting.

In our present measurements we used a radio receiving system very similar to that described in ref. 1. We have six radio receivers, two each at 32 MHz, 44 MHz and 55 MHz, with corresponding bandwidths 3 MHz, 4 MHz and 2 MHz. The antennas are located at a single site 80 m from the centre of the Haverah Park 500 m array. They are oriented to pick up the two horizontal components of the radiation (geomagnetic N–S and E–W) at each frequency. The polar diagram of reception sensitivity in each case consists of a single lobe with its maximum towards the zenith and a null towards the horizon. The receiver outputs are passed through delay cables and linear detectors to separate oscilloscope displays. When the particle array detects a shower the receiver outputs are photographed on a timebase of duration 5 μ s. A time-marker allows one to locate the point on each time base corresponding to the time of arrival of the shower particles.

Over a period of six months, a total of forty-nine radio

showers were identified by the pulses they produced in the 55 MHz receivers. A pulse was accepted as genuine if it occurred at the expected arrival time and was more than twice the amplitude of any other pulse on the trace. We believe all these assignments to be correct; indeed, the results of analysis when rather smaller pulses are also accepted differ only marginally from the ones we shall describe. At 32 MHz and 44 MHz there were only sixteen showers with pulses twice as large as any background pulse at the expected arrival time. Smaller pulses occurring at the expected time were accepted for analysis as well, provided the same shower had produced an unambiguous pulse at 55 MHz. In this way, additional data were obtained from twenty-four showers at the lower frequencies, but we recognize that some of the pulses may be spurious. Accordingly we give less weight to the 32 MHz and 44 MHz data, and concentrate on the 55 MHz results.

The receiver gains were measured with the aid of a pulse-modulated r.f. signal generator and an impulse generator which provided a pulse ~ 10 ns long and of variable amplitude. The observed pulse heights were thereby converted to equivalent voltages at the receiver input terminals. From the calculated antenna gain in the direction of the incoming radio pulse (that is, the direction of the shower axis), and the measured system bandwidth, the voltages were then converted to field strengths per unit bandwidth in the horizontal plane along the geomagnetic N-S and E-W directions. Given these two components of the field and the knowledge that the total electric field vector \mathcal{E} lies in a plane perpendicular to the shower axis, we could thus determine its direction, in other words, the polarization, and also the amplitude of the frequency component \mathcal{E}_ν measured in $\text{V m}^{-1} \text{MHz}^{-1}$ at the frequency ν .

Our chief interest is to relate \mathcal{E}_ν to the relevant parameters of the air shower as measured by the particle array. We expect \mathcal{E}_ν to depend on (a) the primary energy E_p ; (b) the perpendicular distance R from the antenna to the shower axis; (c) the direction of the shower axis. To specify the direction of the shower axis requires two angles. For these we choose θ , the zenith angle, and α , the angle between the shower axis and the geomagnetic field lines. The choice of α , rather than the more familiar azimuthal angle ϕ is made to simplify the analysis, for much evidence already exists to indicate that geomagnetic charge separation is the dominant mechanism of radio emission¹.

With the limited data available, it is not possible to map completely the four-fold dependence of \mathcal{E}_ν on the variables E_p , R , θ and α . Instead, we build up a trial function based partly on the data and partly on theoretical ideas about the dependence of \mathcal{E}_ν on the separate variables; we then look to see how closely the formula as a whole is consistent with all the experimental data.

For all showers, and at all three frequencies (where measurement was possible) the polarization was found to be compatible with that predicted by geomagnetic charge separation. We compared the measured and the predicted ratios of the pulse amplitudes in the horizontal N-S and E-W directions. Only in two cases, with pulses at 32 MHz and 44 MHz that had passed the less onerous acceptance criteria, and were smaller than other background pulses on the trace, was there a numerical discrepancy greater than the error of measurement. We presume that these pulses were in fact spurious.

We tested the other possible radiation mechanisms listed in ref. 1 by the same method, but the correlations so obtained between predictions and measurements were not good. Tentatively, therefore, we assume that the radiation is due wholly to geomagnetic charge separation (in line with at least one theoretical analysis^{9,10}) and accept the implication that \mathcal{E}_ν is proportional to the sine of the angle made by the shower axis with the Earth's field: $\mathcal{E}_\nu \propto \sin \alpha$.

We now consider the dependence of \mathcal{E}_ν on the primary energy E_p . The theory of coherent radiation in its simplest form would require $\mathcal{E}_\nu \propto E_p$, but this may be modified by two secondary effects. As E_p increases, the shower maximum comes closer to the antenna, and on this basis \mathcal{E}_ν could increase more rapidly than E_p . On the other hand, path differences between different parts of the shower and the antenna become progressively greater; this leads to a loss of coherence and to a slower increase of \mathcal{E}_ν with E_p . In an earlier publication¹ we suggested that loss of coherence was the more important effect and that (for E_p between 10^{17} and 10^{18} eV) $\mathcal{E}_\nu \sim E_p^{1/2}$. We now recognize that this earlier analysis was at fault, and that the $E_p^{1/2}$ relation arose from the effect of background noise on the measurements. This reduces the apparent dependence, because for the smaller values of E_p a larger proportion of noise pulses is included among those accepted as genuine. Let us suppose that the dependence of \mathcal{E}_ν on E_p can be expressed by assigning an appropriate value to the exponent β in the equation $\mathcal{E}_\nu \propto E_p^\beta$. We now define a quantity $P = [\mathcal{E}_\nu/E_p^\beta \sin \alpha]$ and call it the normalized pulse amplitude. P will be a function only of R and θ , so if we take showers in a limited range of zenith angles and plot P against R , the points should define a lateral distribution function for the radiation. Implicit in this procedure is the assumption that β is independent of R , in other words, that the lateral distribution has the same shape for different values of E_p . The correctness of this assumption, and of the value of β adopted, can be assessed by the extent to which the experimental points cluster about a unique line for $P = P(R)$.

Our experimental data lead us to adopt a value of the exponent $\beta = 1$. The evidence for this is particularly convincing at $\nu = 55$ MHz and $\theta < 35^\circ$, that is, at the frequency least affected by radio interference and in the zenith angle range where shower analysis from the particle array is at its most reliable. Fig. 1 shows the experimental normalized pulse amplitudes for $\nu = 55$ MHz plotted against R . For showers with $\theta < 35^\circ$ (Fig. 1a) the scatter of the experimental points about the mean line is small. If, for instance, we choose to attribute this scatter entirely to errors in R , then the implied r.m.s. error would be ~ 28 m; such a value is considered to be typical of the actual errors in shower axis location at Haverah Park⁸. The assumption that $\beta = 1$ is therefore compatible with the data.

The showers represented in Fig. 1a have energies E_p between 10^{17} and 10^{18} eV. The only exception is the isolated point at $R = 600$ m which relates to a rare shower with $E_p \sim 1.5 \times 10^{19}$ eV. The points due to the larger showers ($E_p > 3 \times 10^{17}$ eV) are distributed evenly among the other points, and there is no hint of any systematic shift with energy. If, on the other hand, we test the hypothesis $\beta = 0.5$ and plot the quantity $[\mathcal{E}_\nu/E_p^{0.5} \sin \alpha]$ against R , then first, is there an increased scatter about the median line; second, the higher energy showers give points which all fall on the high side of that line. We infer that for E_p between 10^{17} and 10^{18} eV the true value of β is certainly greater than 0.5: we guess it cannot be less than 0.8. The evidence is thus in favour of the value $\beta = 1$ for showers with energies between 10^{17} and 10^{18} eV. Unfortunately, little can be said about β at higher energies, despite the presence of a point in Fig. 1a for $E_p = 1.5 \times 10^{19}$ eV at $R = 600$ m. This point stands well apart from the others, none of which has $R > 250$ m. In Fig. 1a it is normalized just like the others, assuming $\beta = 1$. But a change in the assumed E_p dependence at 1.5×10^{19} eV would merely result in a change in shape of the lateral distribution function beyond 250 m; it leads to no inconsistencies with the data.

Fig. 1b, with data for $\nu = 55$ MHz, $35^\circ < \theta < 50^\circ$, and shower energies $10^{17} - 3 \times 10^{18}$ eV provides supporting evidence in favour of $\beta = 1$, though the scatter is worse than in Fig. 1a. This scatter may be attributed partly to the uncertainties in estimating E_p at large zenith angles, and

partly to changes in the lateral distribution function itself over the rather large range of θ included in the one diagram.

The lateral distribution function for $\nu = 55$ MHz was discussed above in relation to the E_p dependence. We find that for each frequency and zenith angle interval a convenient representation of the distribution is the function $P \propto \exp(-R/R_0)$ where R_0 gives a measure of the lateral spread of the radiation and has different values for different ν and θ . The continuous lines in Fig. 1 are based on the formula with R_0 chosen to give the best fit. For $\nu = 55$ MHz and $\theta < 35^\circ$, $R_0 = (100 \pm 10)$ m. R_0 increases with increasing zenith angle and with decreasing frequency; at 55 MHz and $35^\circ < \theta < 50^\circ$, $R_0 \approx 170$ m, while at 32 MHz and $\theta < 35^\circ$, $R_0 \approx 140$ m. The change is in the direction to be expected from simple geometrical and diffraction considerations, but the measurements are too rough to establish any quantitative relation.

The value of R_0 found for $\nu = 32$ MHz is quite compatible with the data on lateral distribution found by the Moscow group². Their results are expressed as power-law exponents for the dependence of \mathcal{E}_ν^2 on R ; $\mathcal{E}_\nu^2 \propto R^{-n}$, where n varies with R . The measurements of \mathcal{E}_ν^2 are made for different values of R in the same shower, so the Moscow results do not depend, as do ours, on the assumption that the lateral distribution is independent of E_p .

Our measurements show that changes in zenith angle θ have two effects on the radio pulse amplitudes. First (as already discussed) the parameter R_0 in the lateral distribution function increases with θ . Second, there is a general falling off in pulse amplitude by a factor ~ 2 as θ increases from 0° to 50° . As a convenient way of expressing this trend we write $\mathcal{E}_\nu \propto \cos \theta$. Both effects may be interpreted as effects of geometry, related to the increasing distance of shower maximum from the antenna as θ increases.

Because R_0 is frequency dependent, there is no unique dependence of \mathcal{E}_ν on ν ; one must specify the distance from the shower axis, R , at which the spectrum is required. Theoretical calculations^{9,10} suggest that different frequency components of the radiation are associated with shower particles of different kinetic energies E , the higher frequencies depending on the higher energy particles. At high energies (> 100 MeV) scattering is small and the approximation A of cascade theory is valid. For pulses observed on the shower axis the motion of these high energy particles is expected to give a spectrum with \mathcal{E}_ν independent of ν over a wide frequency range. Our present measurements are not precise enough to establish this prediction as a fact, but they are consistent with it. Our measurements at 2 MHz, published previously¹¹, give a considerably larger value of \mathcal{E}_ν at the lower frequency; this too is in line with the theoretical prediction.

The discussion and conclusions reached above can be summarized in a single equation

$$\mathcal{E}_\nu = 25 \left[\frac{E_p}{10^{17}} \right] \sin \alpha \cdot \cos \theta \cdot \exp \left(\frac{-R}{R_0(\nu, \theta)} \right)$$

$\mu\text{V m}^{-1} \text{MHz}^{-1}$

for the ranges E_p , 10^{17} – 10^{18} eV, ν , 32–55 MHz and R , 0–300 m. The parameter R_0 has the value (100 ± 10) m for $\nu = 55$ MHz and $\theta < 35^\circ$. R_0 increases by a factor ~ 2 as θ increases from 0° to 50° and as ν decreases from 55 MHz to 32 MHz. Extrapolation beyond any of these

limits is hazardous; in particular we expect that \mathcal{E}_ν may increase less rapidly with E_p as E_p increases beyond 10^{18} eV.

Finally, we wish to emphasize that the scatter shown in Fig. 1 is representative of all showers recorded by the Haverah Park array, and not of a small sub-set of "radio showers". We re-examined the particle and radio records of some 200 showers which had not given a detectable radio pulse. In every case the expected pulse amplitude as predicted from the particle data by the equation above was below the acceptance threshold. The most common reason for this was that with $E_p \sim$ a few times 10^{17} eV the distance R was in excess of 300 m. Thus Fig. 1 indi-

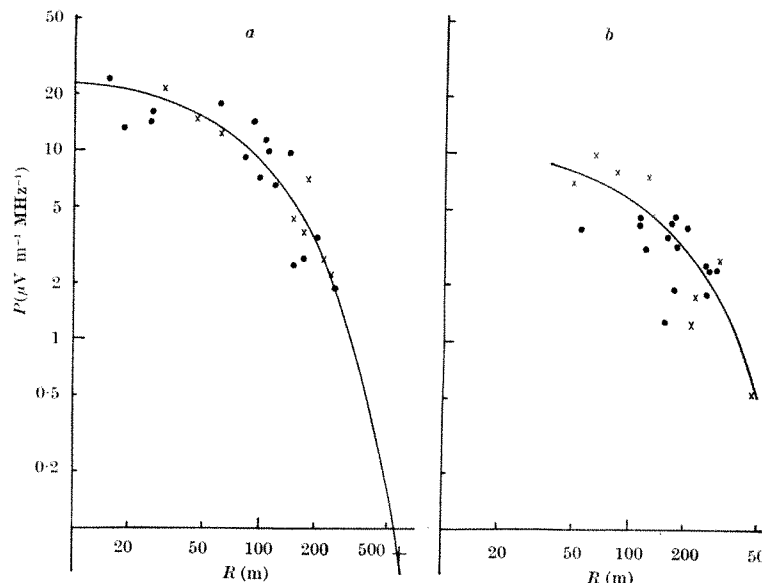


Fig. 1. Normalized radio pulse amplitude, P , at 55 MHz as a function of distance R from the shower axis. The normalization is carried out assuming the amplitude is proportional to the total shower energy E_p and to the sine of the angle made by the shower axis with the Earth's magnetic field. The ordinate gives the field strength in $\mu\text{V m}^{-1} \text{MHz}^{-1}$ for a shower of total energy 10^{17} eV moving perpendicular to the Earth's field. a, Zenith angle $\theta < 35^\circ$; ●, shower energy $10^{17} < E_p < 3 \times 10^{17}$ eV; x, shower energy $3 \times 10^{17} < E_p < 10^{18}$ eV; +, $E_p = 1.5 \times 10^{19}$ eV. b, Zenith angle $35^\circ < \theta < 50^\circ$; x, showers with $35^\circ < \theta < 42^\circ$; ●, showers with $42^\circ < \theta < 50^\circ$.

cates, to within the accuracy of measurement, a unique functional relationship between radio pulse amplitude and the shower particle parameters.

We thank Messrs D. Pearce, J. Prah, D. Andrews and all our colleagues at Haverah Park for their helpful cooperation throughout this work.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Search for Millimetre-wave Emission from Blue Stellar Objects

ACCORDING to the list of Burbidge and Burbidge¹, there are now 30 "radio-quiet" quasi-stellar objects, or QSOs (hereinafter called blue stellar objects, or BSOs) with measured redshifts. The term "radio-quiet" here means that they were selected optically and have not been identified in any general survey of radio sources. A number of BSOs have been examined by several different observers for possible radio emission on wavelengths from 94 to 2 cm, nearly always with negative results. In spite of this lack of success, a new survey of BSOs at millimetre wavelengths was felt worthwhile. No extensive surveys have been made at less than 11 cm wavelength; Kellermann and Pauliny-Toth² observed only three BSOs at 2 cm, with no detections. Many known QSOs have high surface brightness and hence positive spectral indices at short centimetre wavelengths³; thus BSOs might well be detectable only at short wavelengths.

The present observations at 9.5 and 3.5 mm (31.5 and 85.3 GHz) were made in December 1969 and January 1970 with the National Radio Astronomy Observatory 36 foot dish at Kitt Peak, Arizona. The receiver r.m.s. output fluctuations for 30 s of ideal integration time were equal to 2.8 flux units (f.u.) at 3.5 mm wavelength, and 1.9 f.u. at 9.5 mm (1 f.u. = 10^{-26} W/m² Hz). Dicke-switched dual feed systems were used, producing two antenna beams separated by about three half-power beamwidths. Observations consisted of 30 s integrations, with the source alternating in the two beam positions. The integrations were continued until the r.m.s. noise level at each source position was less than 0.3 f.u. The data were taken over a wide range of hour angle, while the antenna polarization was fixed horizontally; thus the data are averages over a range of position angles. Flux density calibrations were made by observing the standard sources Saturn, Jupiter and DR 21.

The sources observed are listed in Table 1. In no case was a deflexion more than three times the 0.3 f.u. r.m.s. level observed, so that an upper limit of 1.0 flux units at both 9.5 and 3.5 mm wavelength can be placed on all sources. The wavelengths and flux levels reached by previous observers are also included in Table 1. The only definite detection is PHL 1222 by Braccisi⁴ at 11 cm. Lang and Terzian¹⁰ reported detection of seventeen BSOs at the 0.3 or 0.4 f.u. level, but their identifications have been questioned by Grueff⁸, who used a smaller antenna beam and found that most of the radio and optical positions he observed did not agree within the estimated errors.

Most of the BSOs in Table 1 have now been surveyed at a number of wavelengths from 94 cm to 3.5 mm, with no detectable emission. A possibility which the earlier observations did not exclude was that BSOs are compact

and have a high radio surface brightness, hence remaining synchrotron self-absorbed to very short wavelengths. QSOs with compact components of this type are known to exist—for example, 3C345, with a peak flux density of 20 f.u. at 8.5 mm, and 3C273, with a peak flux of 30 f.u. at 10 mm (ref. 3). The present observations show that any such components for BSOs must either be less than 1.0 f.u. peak or have a cut-off wavelength $\lesssim 3.5$ mm, which implies a radio surface brightness about ten times that of any QSO. Achieving such a high surface brightness with a brightness temperature less than 10^{12} K (the maximum allowed for a reasonable lifetime with inverse Compton scattering) would require a magnetic field $\geq 10^{-2}$ G, compared with estimates of $\sim 10^{-4}$ G for known QSOs³.

Table 1. BSOs OBSERVED AT 9.5 AND 3.5 MM WAVELENGTH

Source	Previous observations	Source	Previous observations
PHL 3632	—	BSO 2	KPT, LT, BFGV, BLS
PHL 938	LT, GR	B 114	LT, BLS
PHL 3375	B, FGF, LT	B 154	LT, BLS
PHL 1027	B, FGF, LT, GR	B 194†	LT, G, BLS
PHL 3424	B, FGF, LT	B 189	LT, BLS
PHL 1070	B, FGF, LT	B 201	LT, G, BLS
PHL 1072	B, FGF, LT	BSO 6	LT, G, BFGV, BLS
PHL 1186	B, FGF, LT	B 264	LT, G, BLS
PHL 1194	B, FGF, LT, GR	B 234	LT, G, BLS
PHL 1222*	B, FGF, LT, GR	B 340	LT, BLS
PHL 1226	B, FGF, LT	B 312	LT, BFGV, BLS
QS1108+285	LT	BSO 7	BFGV, BLS
Ton 1530	LT	BSO 8	BFGV, BLS
Ton 1542	LT	BSO 11	KPT, LT, G, BLS
BSO 1	KPT, SW, LT, BFGV, BLS	RS 13	LT, G
B 46	LT, BLS	Ton 256	KPT, SW, LT, G

* Detected by B; 0.1 f.u. at 11 cm.

† LT report 1.2 f.u., but BLS report ≤ 0.5 f.u.

Sources listed in order of right ascension. The upper limit to the current observations is 1.0 flux unit at both wavelengths. Other observers, wavelengths, and upper limits are:

B, Braccisi⁴ (11 cm, 0.075 f.u.).

BLS, Braccisi, Lynds, and Sandage⁵ (75 cm, 0.5 f.u.; 11 cm, 0.2 f.u.).

BFGV, Braccisi *et al.*⁶ (75 cm, 0.75 f.u.).

FGF, Fanti-Giovannini and Fanti⁷ (75 cm, 0.25 f.u.).

G, Grueff⁸ (75 cm, 0.2 f.u.).

GR, Grewing and Ristow⁹ (11 cm, 0.4 f.u.).

KPT, Kellermann and Pauliny-Toth² (6 cm, 0.10 f.u.; 2 cm, 0.2 f.u.).

LT, Lang and Terzian¹⁰ (94 cm, 0.3 f.u.).

SW, Shapiro and Weinreb¹¹ (3.75 cm, 0.5 f.u.; 2 cm, 2.0 f.u.).

The upper limits quoted are three times the r.m.s. noise if limits are not stated specifically in the original paper.

A comparison of the optical properties of QSOs and BSOs shows no clear systematic difference that might explain the lack of radio emission. The list of Burbidge and Burbidge¹ shows few BSOs with $m_r \leq 16.8$, but above this limit the ratio of BSOs and QSOs in any given visual magnitude range is roughly constant. In any case, Longair and Scheuer¹² found no correlation between optical and radio magnitudes of 75 QSOs. They concluded that there was probably a wide dispersion in both radio and optical luminosities of QSOs. Concerning redshifts, 50 per cent of the BSOs have $z \leq 0.5$, compared with 24 per cent of the QSOs; but Bolton¹³ has recently found a lack of correlation between redshift and flux density at 2,700 MHz for QSOs, even for flat spectrum sources (spectral index ≤ -0.7).

It appears likely that BSOs are similar to QSOs but have sufficiently low radio emission to have escaped detection. This conclusion is not unexpected considering the relative luminosities of QSOs and galaxies. Heeschen¹⁴ has shown that QSOs and galaxies (particularly radio galaxies) form a continuous sequence on a plot of absolute radio luminosity against surface brightness. The observed range of QSO luminosities, however, is only 10^{43} to 10^{45} erg/s, compared with 10^{37} to 10^{39} erg/s for spiral galaxies, $10^{38.5}$ to $10^{41.5}$ erg/s for E and SO galaxies, and 10^{41} to 10^{45} erg/s for radio galaxies¹⁵. It thus seems

reasonable that BSOs are not a separate class of stellar objects, but are QSOs with small intrinsic radio luminosity.

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Evidence for an Anisotropy in the Cosmic Ray Signal

COSMIC rays are one of the chief constituents of the physical universe but their origins are still not understood. Anisotropies in the cosmic ray signal should provide information toward the solution of these problems. This report presents evidence for an anisotropy in the cosmic rays centred around 0900 h sidereal time and between $+30^\circ$ and $+60^\circ$ declination.

The anisotropy has been observed with a cosmic ray telescope 30 miles south of Plattsburgh, New York (approximately 45° N, 74° W, and 400 feet above sea level).

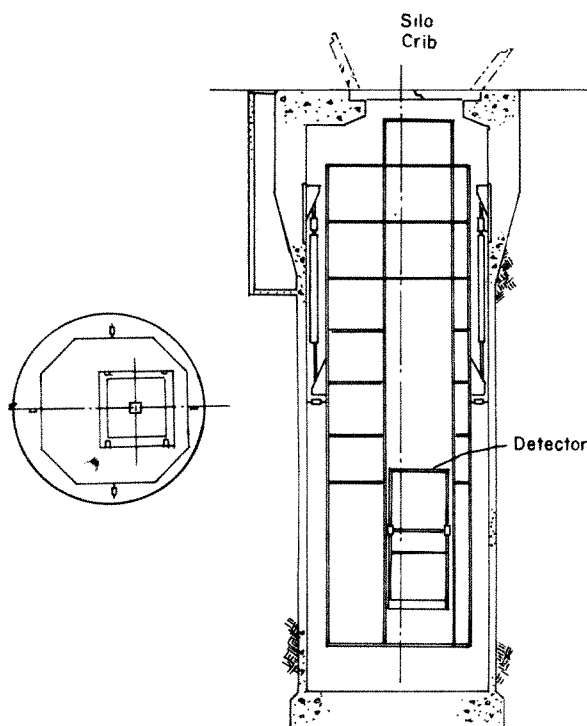


Fig. 1. Sketch of silo laboratory indicating the position of the detector.

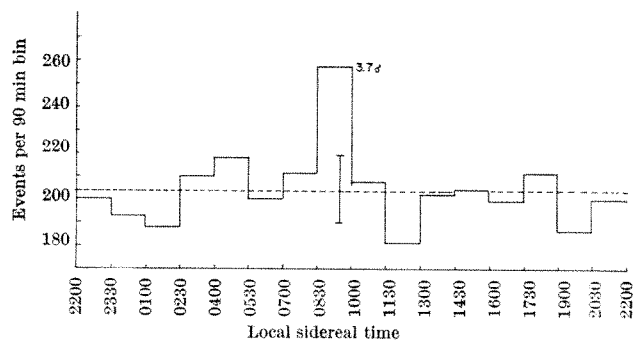


Fig. 2. Sidereal time histogram. Number of event per 90 min bin versus local sidereal time. Data were collected from August 1967 to June 1968 using a 50 gallon target. Over 3,000 events are plotted.

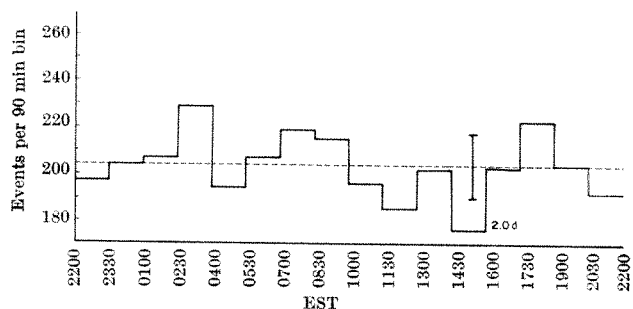


Fig. 3. Eastern standard time histogram. Number of events per 90 min bin versus eastern standard time. Data were collected from August 1967 to June 1968 using a 50 gallon target. Over 3,000 events are plotted.

The data consist of the clock times of muon events in a liquid scintillator and were collected between August 1967 and June 1968, and from December 1968 to June 1969 (Figs. 2, 5). This work is a continuation of earlier investigations with similar detectors.

The experiment is concerned with muons of moderate energy (less than 200 MeV) which appear and decay in the detector. The telescope consists of an abandoned Atlas F missile silo and a liquid scintillation counter surrounded by an anti-coincidence shield of plastic scintillator. The detector is about 120 feet below ground level. The experimental arrangement is similar to earlier experiments at the Catholic University, Washington, DC, the most important difference being the collimating effects of the silo (Fig. 1), approximately 180 feet deep and 50 feet in diameter. Directly above the detector, at a height of about 120 feet, are concrete doors two feet thick. The resolution of the telescope is estimated to be $\pm 15^\circ$ in right ascension and declination. The part of the sky visible to the detector extends approximately from $+30^\circ$ to $+60^\circ$ declination, and is swept once every 24 h. From August 1967 to June 1968, fifty gallons of liquid scintillator were used as the detector target. The liquid was contained in a 'Plexiglass' box 48 inches by 24 inches by 12 inches filled to within 1 inch of the top. From December 1968 to June 1969 eighty gallons of liquid scintillator, contained in a plexiglass box 48 inches by 24 inches by 24 inches filled to within 6 inches of the top, were used as the target. Thus there were two independent runs under somewhat different detector configurations. Moderate energy muons which appear and decay in the detector, and which satisfy the anti-coincidence requirement, are presumed to have been produced by a neutral particle in the cosmic rays.

The 'Plexiglass' tank is viewed by two 58 AVP photomultiplier tubes and is surrounded on all sides by an anti-coincidence shield of 3/4 inch plastic scintillator. The plastic scintillator is viewed by one 58 AVP and twenty-six XP1000 photomultiplier tubes. The two 58 AVP tubes viewing the liquid scintillator are run in coincidence with each other and in anti-coincidence with the twenty-seven photomultiplier tubes attached to the anti-coincidence shield.

Data are collected by a camera attached to an oscilloscope which are both triggered whenever an event in the detector has satisfied the coincidence and anti-coincidence requirements. A particle satisfying the anti-coincidence condition generates an electronic gate 6 μ s long which is delayed 0.3 μ s relative to the incident particle. If the incident particle decays during the time that the gate is open ($\mu \rightarrow e + V_e + V_\mu$) the oscilloscope and camera are triggered. The data consist of two pulses, the incident and the decay particles and EST of events. So far more than 8,000 muon-like events have been observed. The signal rates for the two runs were, on the average, 0.75 per hour and 1.75 per hour respectively.

The data are printed out in ninety-six 15 min sidereal and EST bins. The events in each bin are then combined into 90 min bins and histograms of the number of events per 90 min bin versus sidereal and EST are plotted. An event is assumed to be a muon and its decay electron.

The equipment is checked periodically by taking the rates of ordinary cosmic ray muons satisfying the coincidence and delayed coincidence condition with the anti-coincidence turned off. The background coincidence rates for the two runs averaged approximately 600 c.p.m. and 650 c.p.m. and the delayed coincidence, the number of stopping muons in the detector from ordinary cosmic rays, averaged approximately 1.5 per min and 3.0 per min respectively for the two detector configuration.

Data were collected with some shut downs, from August 1967 to June 1968 (Figs. 2, 3 and 4). There is a peak of 3.7 standard deviations in Fig. 2 between 0830h and 1000h local sidereal time. The probability of such a peak occurring by chance is less than one in four thousand (0.025 per cent probability of randomness). The same data plotted as a function of EST shows no peaks greater than 2.0 standard deviations which is to be expected because the sidereal clock gains roughly four min a day on the solar clock. In Fig. 4 the data are plotted in 60 min bins as a function of sidereal time. A peak of 3.9 standard deviations occurs around 0900 h local sidereal time. The probability of randomness is less than 0.01 per cent—that is less than 1 in 10,000.

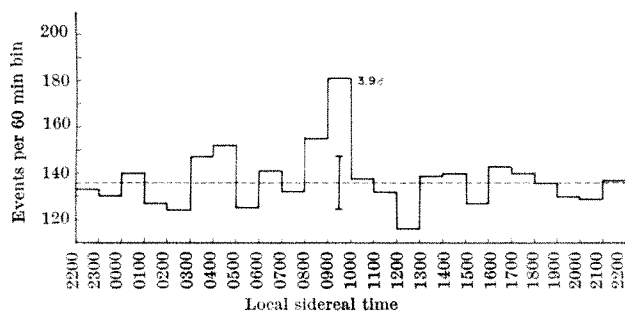


Fig. 4. Sidereal time histogram. Number of events per 60 min bin versus local sidereal time. Data were collected from August 1967 to June 1968 using a 50 gallon target. Over 3,000 events are plotted.

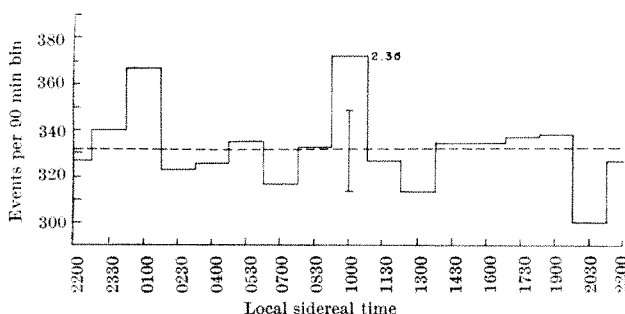


Fig. 5. Sidereal time histogram. Number of events per 90 min bin versus local sidereal time. Data were collected from December 5, 1968, to June 1, 1969, using an 80 gallon target. Over 5,000 events are plotted.

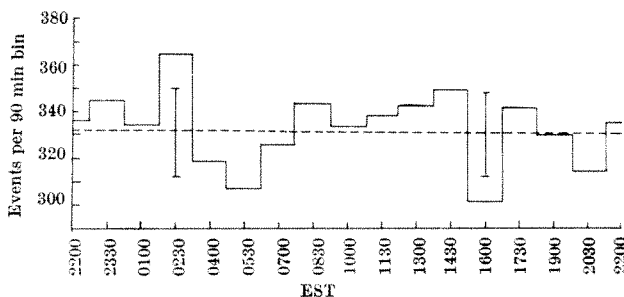


Fig. 6. Eastern standard time histogram. Number of events per 90 min bin versus eastern standard time. Data were collected from December 5, 1968, to June 1, 1969, using an 80 gallon target. Over 5,000 events are plotted.

The second run began in December 1968. Fig. 5, the sidereal histogram of the number of events per 90 min bin, has a peak of 2.3 standard deviation between 0915 h and 1045 h local sidereal time. The same data plotted as a function of EST (Fig. 6) have no peaks greater than 1.8 standard deviations.

Because the anti-coincidence factor is estimated to be 500 to 1, one cannot rule out leakage muons from the cosmic ray background. The estimate is based on the measurement of the anti-coincidence factor using an external detector placed above and outside the box containing the telescope detector. This means that about 1/3 of the events are due to leakage muons from the cosmic ray background and could be responsible for the sidereal effect. The observed muon signal does indicate a strong dependence on sidereal time and the effect has persisted in the same area of the sky for two consecutive years and under somewhat different detector configurations. The reality of the anisotropy seems to be well established.

Plans are in progress to move the detector fifty feet deeper into the silo which should improve the resolution of the telescope. We are also making efforts to determine whether the muons are leakage muons or are produced in the detector by a neutral particle in the cosmic rays.

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Compositions of Garnets in Interstellar Dust

HUFFMAN¹ has shown that absorption bands in the optical spectra of the supernova in NGC 4496 correlate well with d-d bands of Fe³⁺ in yttrium iron garnet and Fe²⁺ in terrestrial almandine, Fe₃²⁺Al₂³⁺Si₃O₁₂. I have suggested² that the 4430 Å band in supernova spectra is a result of Fe³⁺ in a silicate matrix, the band being reminiscent of that marking the ⁶A₁(S)→⁴A₁E(G) transition

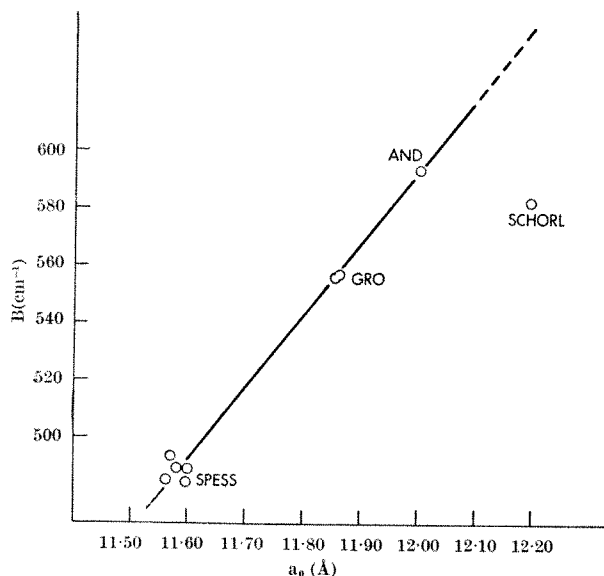


Fig. 1. A plot of B against a_0 for a series of garnets. SPESS, spessartine; GRO, grossular; AND, andradite; SCHORL, schorlomite.

in octahedral Fe^{3+} in andradite garnet, $\text{Ca}_3\text{Fe}_2\text{Si}_3\text{O}_{12}$. It is likely that interstellar garnets are silicate-based, and here I propose approximate formulae for these garnets based on the identification of two interstellar absorption bands, the energies of which can be related to garnet compositions.

The transitions ${}^6\text{A}_1 \rightarrow {}^4\text{A}_1, {}^4\text{E}(\text{G})$ and ${}^6\text{A}_1 \rightarrow {}^4\text{E}(\text{D})$ in Mn^{2+} and Fe^{3+} are field-independent^{3,4}, and the corresponding absorption bands are relatively sharp^{4,5}. The energies of these transitions, ν_1 and ν_2 respectively, are related to the Racah interelectronic repulsion parameters, B and C , by

$$\nu_1 = 10B + 5C \text{ and } \nu_2 = 17B + 5C$$

from which B and C can be measured if ν_1 and ν_2 can be determined from spectra⁵. The B values for metal ions in crystals are smaller than for free ions, and Jorgensen^{6,7} attributes this decrease in B to covalent bonding, in which metal d-electrons occupy a greater volume encompassing metal and anion. Jorgensen proposed that for a given cation, B is a measure of covalency: the smaller B is, the greater is the covalency. B values derived by Keester and White⁵ for Mn^{2+} in oxides and silicates show that there is reasonable correlation between B and covalency.

Table 1. SPECTRAL DATA FOR OCTAHEDRAL Fe^{3+} IN SILICATES (ν_1 , ν_2 AND B IN cm^{-1})

Silicate	Cube ion	Oct. ion	ν_1^*	ν_2	B
Spessartine	$\text{Fe}^{2+}\text{Mn}^{2+}$	Al^{3+}	23500	26900	490
Grossular	Ca^{2+}	Al^{3+}	23100	27000	555
Andradite	Ca^{2+}	Fe^{3+}	22850	26950	590
Schorlomite	Ca^{2+}	Fe^{3+}	22900	27000	590
Muscovite			22600	27300	670
Kyanite			22900	27000	585

* Except for muscovite, indicates average energy of ${}^4\text{A}_1(\text{G})$ and ${}^4\text{E}(\text{G})$ levels. B (free ion) = 930 cm^{-1} (ref. 9).

Recently, I determined⁸ B values for octahedral Fe^{3+} ions in a number of silicates, including garnets. Values of ν_1 , ν_2 and B are listed in Table 1, while a plot of B against a_0 (the cell edge) for garnets is shown in Fig. 1. Absorption bands marking ν_1 and ν_2 in grossular and andradite were identified earlier¹⁰, whereas bands corresponding to ν_1 and ν_2 for a spessartine are shown in Fig. 2. The two sharp peaks at $23,300 \text{ cm}^{-1}$ and $23,700 \text{ cm}^{-1}$

mark transitions to ${}^4\text{A}_1(\text{G})$ and ${}^4\text{E}(\text{G})$ levels separated by 400 cm^{-1} . Both components are also observed in andradite and schorlomite spectra, but the $22,700 \text{ cm}^{-1}$ band is much the stronger. The estimated error in B is $\pm 10 \text{ cm}^{-1}$.

Fe^{3+} -O distances will certainly increase with increasing a_0 , hence the corresponding decreasing trend in covalency (rising B) can be attributed to poorer d-orbital overlap. The parent Al-O distances in pyrope ($a_0 = 11.46 \text{ Å}$)¹¹ and grossular ($a_0 = 11.85 \text{ Å}$)¹² are 1.89 Å and 1.945 Å , respectively.

Schorlomite¹³ is a high-Ti andradite in which each octahedral cation has an average of 1.5 Al^{3+} or Ti^{4+} ions in the next-nearest-neighbour tetrahedral sites. These latter ions form bonds which are less covalent than those of Si, and hence nett charge on the oxygens increases and octahedral- Fe^{3+} -O bonds become more covalent. Substitution of Si ions therefore lowers the value of B .

Table 2. COMPARISON OF BAND ENERGIES IN Fe^{3+} SPECTRA (cm^{-1})

Andradite (oct. Fe^{3+})	Kimzeyite (oct. + tet. Fe^{3+})	Supernova 4496	Fe^{3+} coord. (this work)
26950*		26300	OCT.
	24100	24390	TET.
22700*	22700	22600	OCT.
		20600	TET.
	20600	20400	TET.
		19600 (Fe^{2+})	
		17500 (Fe^{2+})	
16700*	16700	16100	OCT.

* In increasing energy, mark transitions to ${}^4\text{T}_2(\text{D})$, ${}^4\text{A}_1, {}^4\text{E}(\text{G})$ and ${}^4\text{E}(\text{D})$ levels respectively.

The absorption spectrum of the garnet kimzeyite¹⁴, in which Fe^{3+} ions are located in both octahedral and tetrahedral sites, shows two bands in addition to the octahedral Fe^{3+} bands of andradite (Table 2). These bands, at $20,600 \text{ cm}^{-1}$ and $24,100 \text{ cm}^{-1}$, very likely mark transitions to the ${}^4\text{T}_2(\text{G})$ and ${}^4\text{A}_1, {}^4\text{E}(\text{G})$ levels in tetrahedral Fe^{3+} , and correspond to the $20,300 \text{ cm}^{-1}$ and $22,700 \text{ cm}^{-1}$ bands observed in spectra of tetrahedral Fe^{3+} in phlogopite¹⁵. A band at $\sim 16,700 \text{ cm}^{-1}$ marking transitions to ${}^4\text{T}_2(\text{G})$ levels is a characteristic feature of octahedral- Fe^{3+} spectra^{10,16}.

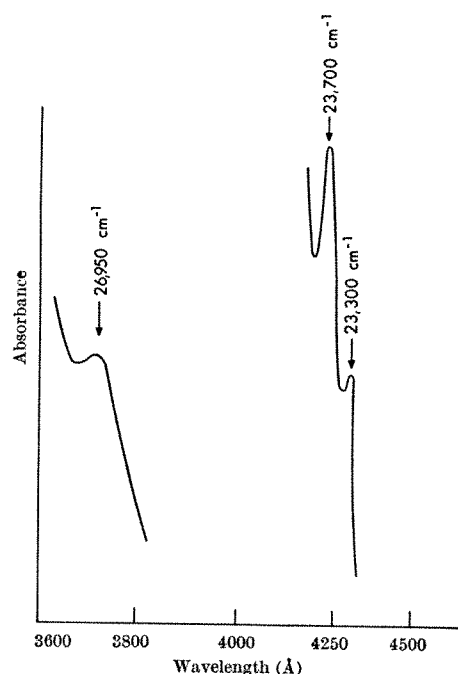


Fig. 2. Parts of absorption spectrum of a spessartine from Ceara, Brazil, showing bands corresponding to ν_1 and ν_2 .

The spectrum of SN-NGC-2713 (ref. 17), presented in Fig. 3, is similar to that of SN-NGC-4496 (ref. 18) except that a band is observed in the latter spectrum at 3800 Å and that the band at 4870 Å in the former is resolved into two components at 4850 Å and 4900 Å in the latter. By analogy with the bands in natural garnets, I would assign the 16,100 cm⁻¹ (6200 Å), 22,600 cm⁻¹ (4430 Å) and 26,300 cm⁻¹ (3800 Å) bands to transitions to the ⁴T₂(G), ⁴A₁E(G) and ⁴E(D) levels in octahedral Fe³⁺. The 20,600 cm⁻¹ (4850 Å), 20,400 cm⁻¹ (4900 Å) and 24,400 cm⁻¹ (4100 Å) bands can be assigned to tetrahedral Fe³⁺. The B-value for octahedral Fe³⁺ based on $\nu_1 = 22,600$ cm⁻¹ and $\nu_2 = 26,300$ cm⁻¹ is 535 cm⁻¹. Because the replacement of Si by other cations will increase rather than decrease the covalence of octahedral Fe³⁺-O bonds, it is likely that the interstellar garnet is an andradite in which a significant proportion of Si⁴⁺ ions have been replaced by Fe³⁺ and possibly Al³⁺ ions. The envelope marking transitions to the ⁴A₁E(G) levels in supernova spectra is most similar in energy and shape to that of an andradite or a Si-substituted andradite, while the band half-widths measured earlier² imply substitution of Si by other larger cations. It would therefore appear that the Fe³⁺ ions are located in octahedral and tetrahedral sites in the same crystal.

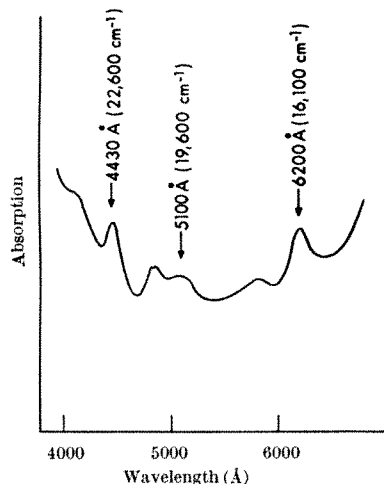
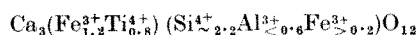


Fig. 3. Spectrum of supernova in NGC 2713 in optical region¹⁷.

There is no continuous change in composition between almandine-type and andradite-type garnets¹⁹. Huffman¹ has compared the interstellar bands at 19,600 cm⁻¹ (5100 Å) and 17,500 (5700 Å) to spin-forbidden bands of 8-coordinate Fe²⁺ in almandine. The extinction coefficient of the 19,700 cm⁻¹ band in terrestrial almandine is approximately five times smaller than that of the 22,700 cm⁻¹ band in andradite, which in turn is ten times smaller than that of the 22,700 cm⁻¹ band in schorlomite²⁰. Hence, supernova in NGC 2713 and NGC 4496 contain considerably more almandine than andradite-type garnet.

Based on ionic radii and known chemistry in oxygen and garnet systems²¹, Al³⁺ is most likely to substitute for Si⁴⁺, followed by Fe³⁺ and then Ti⁴⁺. Assuming a 25 per cent deficiency of Si⁴⁺ ions in tetrahedral sites and that there are no Al³⁺ ions in octahedral sites, an approximate formula is Ca₃(Fe³⁺Ti⁴⁺)₂(Si_{1.25}Al_{0.75}Fe³⁺)O₁₂. To balance charges, 0.8 Ti⁴⁺ ions are required in octahedral sites, while band intensities indicate that the ratio of tetrahedral-Fe³⁺ : octahedral-Fe³⁺ is not likely to be as low as 1:10. Hence the formula reduces to



If the current assignments of bands in supernova spectra are correct, interstellar dust contains two garnets,

one of the almandine-type and the other a substituted andradite with appreciable amounts of Fe³⁺ and, possibly, Al³⁺ in Si⁴⁺ sites.

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Five New Pulsars

FIVE new pulsars have been discovered in a systematic search at low galactic latitudes, using the Mark I radio telescope at Jodrell Bank, and a radio frequency of 408 MHz. A total of 431 independent points were observed (many of them more than once) at integral degrees of galactic latitude and longitude, including all such points with latitude $-1, 0$, and $+1^\circ$ and longitude $11^\circ \leq l \leq 125^\circ$. The beamwidth of the telescope to half power was $0^\circ.8$.

The system was designed to detect periodic signals in the range of periods $0.2 < P < 3.6$ s. The outputs of two receivers, each with 4 MHz bandwidth and detecting the two hands of circular polarization, were combined, and the detected signal was sampled digitally every 50 ms over a period of 13 m 39 s, that is for 2^{14} samples. Periodic signals were found by a process amounting to cross correlation of this digital signal with pulse trains of duty cycle 25 per cent. The system was therefore equally sensitive to pulsars with a large range of dispersion measure, which would be expected to lengthen the pulses in the wide receiver bandwidth. For pulsars with dispersion measure up to $500P$ cm⁻³ pc, and lying close to the beam centre, the limiting flux density is 0.02×10^{-26} W m⁻² Hz⁻¹, and it is unlikely that any pulsar stronger than this limit within $10'$ arc of the positions observed has escaped detection.

The new pulsars have been studied at 406, 408, 410 and 610 MHz to obtain information on the dispersion measure, pulse shape and period. The data and standard errors are given in Table 1. The barycentric periods have been calculated using the quoted positions, and the errors in period refer only to the uncertainty in timing measurements. Two of the new pulsars, JP 2003 and JP 2319, have periods in excess of 2 s; only one other known pulsar (NP 0527) has a longer period. JP 2003 has an unusually short pulse, the equivalent width (pulse energy divided by peak flux density) being only 1 per cent of the period. JP 2319 is situated only $1^\circ.5$ from the supernova remnant

Cas A, but it is unlikely that the objects are physically associated; the age and distance of Cas A (300 yr, 3.5 kpc) would imply that the pulsar was ejected from the supernova at the velocity of light.

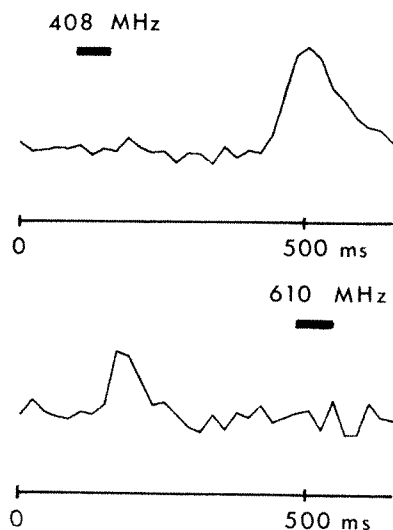


Fig. 1. A computer graphical output showing the integrated pulse profiles of JP 1858 at 408 MHz and 610 MHz. The difference in position of the pulse is due to the dispersion and corresponds to 290 ms plus an integral number of periods. The effective resolution (due to time constant, sample length and dispersion in the receiver bandwidth) is indicated on each trace by a horizontal bar. The unusual width and extended trailing edge of the pulse at 408 MHz is thought to be caused by scintillation broadening.

The most interesting of the new pulsars is JP 1858, which has the highest dispersion measure of any known pulsar. The distance, based on this dispersion measure and an assumed mean electron density $\bar{n}_e = 0.05 \text{ cm}^{-3}$ is 8 kpc. It thus lies at about 5 kpc from the galactic centre, presumably in an inner spiral arm. Fig. 1 is a

a consequent scatter in the arrival time of the pulse at a single frequency. A simplified argument suggests that scintillation should produce an exponential tail to the observed pulse with a decay time constant that increases with the square of the dispersion measure and the fourth power of the wavelength. A similar effect has been reported³ for the Crab pulsar (NP 0532) where the extremely short natural pulse makes scintillation broadening apparent at a moderate dispersion measure. Assuming the scale of the effect indicated by these two pulsars is characteristic of the interstellar medium in general, scintillation broadening should be detectable by measurement of high dispersion pulsars at a number of frequencies, and should provide valuable further information on the clumpiness of the ionized interstellar medium.

Scintillation broadening can be expected to limit the sensitivity of searches for high dispersion pulsars, particularly those of short period. As an example it is interesting to consider the effect on the Jodrell Bank and Molonglo searches at 408 MHz (which together have discovered 38 of the 55 known pulsars). The pulse shapes of JP 1858 indicate that at 408 MHz the broadening is given by

$$t \text{ (ms)} \approx \left(\frac{dm}{50} \right)^2$$

where dm is the dispersion measure in $\text{cm}^{-3} \text{ pc}$. On this basis, the sensitivity of 408 MHz searches would deteriorate rapidly for dispersion measures greater than 300 or $1,000 \text{ cm}^{-3} \text{ pc}$ for periods of 0.1 s or 1.0 s respectively. Hence in searches carried out so far at 408 MHz, scintillation broadening is probably less important than widening of the pulse by dispersion in the receiver bandwidth as a cause of loss of high dispersion short period pulsars. As scintillation broadening increases with the square of the dispersion measure, however, and cannot be corrected for by techniques analogous to "dispersion removing", it may prove a serious obstacle to extending pulsar searches to remote regions of the Galaxy.

Table 1

	JP 1845	JP 1858	JP 1953	JP 2003	JP 2319
$\alpha 1950$	$18^{\text{h}}45^{\text{m}}10^{\text{s}} \pm 45^{\text{s}}$	$18^{\text{h}}58^{\text{m}}40^{\text{s}} \pm 45^{\text{s}}$	$19^{\text{h}}53^{\text{m}}00^{\text{s}} \pm 60^{\text{s}}$	$20^{\text{h}}03^{\text{m}}00^{\text{s}} \pm 60^{\text{s}}$	$23^{\text{h}}19^{\text{m}}15^{\text{s}} \pm 90^{\text{s}}$
$\delta 1950$	$-4^{\circ}0'0'' \pm 0^{\circ}2'$	$+3^{\circ}4'0'' \pm 0^{\circ}2'$	$+29^{\circ}2'0'' \pm 0^{\circ}2'$	$+31^{\circ}5'0'' \pm 0^{\circ}2'$	$+60^{\circ}0'0'' \pm 0^{\circ}1'$
l	29°	37°	66°	69°	112°
b	-1°	$-0^{\circ}7'$	$+0^{\circ}7'$	0°	$-0^{\circ}7'$
P s (bary centric)	$0.597731 \pm 2 \mu\text{s}$	$0.655444 \pm 1 \mu\text{s}$	$0.426676 \pm 1 \mu\text{s}$	$2.111206 \pm 1 \mu\text{s}$	$2.256483 \pm 1 \mu\text{s}$
Date of observation	1970 June 15	1970 June 16	1970 June 16	1970 June 18	1970 May 27
Dispersion measure $\text{cm}^{-3} \text{ pc}$	141.9 ± 0.6	402 ± 2	~ 20	225 ± 10	96 ± 3
Equivalent width at 408 MHz ms	20 ± 5	120 ± 10	20 ± 5	25 ± 5	140 ± 30
Mean flux density at 408 MHz $\text{W m}^{-2} \text{ Hz}^{-1} \times 10^{-26}$	$0.04 \pm 30 \text{ per cent}$	$0.03 \pm 30 \text{ per cent}$	$0.02 \pm 30 \text{ per cent}$	$0.02 \pm 30 \text{ per cent}$	$0.02 \pm 30 \text{ per cent}$

graphical output from the computer showing 4 h 45 m integration of pulses from JP 1858 at 408 and 610 MHz. The difference in position of the pulse on the two traces is due to dispersion, and corresponds to 290 ms plus 8 complete periods. It will be seen that the observed pulse is considerably longer at 408 MHz than at 610 MHz. At 408 MHz the bandwidth used in this measurement was 1.1 MHz giving a dispersion widening of 55 ms. At 610 MHz the 4.3 MHz bandwidth gives a dispersion widening of 64 ms. At each frequency the analogue time constant was 10 ms and the digital sample interval was 20 ms. Thus the effective resolution at 408 MHz is marginally better than at 610 MHz.

The natural pulse width of typical pulsars depends very little on frequency^{1,2} and we suggest that this unusually wide asymmetric pulse at 408 MHz is a result of scintillation broadening made apparent by the very large dispersion measure. Irregularities in the ionized interstellar medium give rise to multiple path propagation and

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Lake Toba, Sumatra, and the Origin of Tektites

KAYSING suggested recently¹ that Lake Toba, Sumatra, Indonesia, was a possible source area for the Australian group of tektites. This suggestion was based on extrapolation from the observation of McColl and Williams² that the "majority of australite localities in south central Australia are concentrated along a line extending north-westwards.... A second subparallel line may exist in the west of South Australia, separated from the main line by an extensive tract of australite-deficient country". They consider that these lines represent primary distribution patterns, and Kaysing¹ suggests that suitable assumptions about ejection angles and allowance for the Earth's rotation "brings the intersection of the infall lines to Lake Toba".

Lake Toba, the largest lake in Indonesia, is one of the largest terrestrial features ascribed to volcanic action. The lake is 87 km long (the depression within which the lake occurs is 100 km long) and 31 km wide³. It is surrounded by a vast deposit of acid volcanic material, interpreted as welded tuff or ignimbrite, covering an area of 25,000 square km, with a volume of 2,000 cubic km⁴. This forms one of the largest occurrences of this type of rock and is matched in volume only by the large welded tuff sheets of the central North Island of New Zealand, the type locality for ignimbrite⁵. The consensus among geologists who have investigated Lake Toba is that it is a volcano-tectonic depression, which subsided after paroxysmal eruptions (Nuée ardente type) of acidic material from an underlying magma chamber. Westerveld⁴, in particular, is convinced of the "great parallelism between volcanic phenomena on Sumatra and in Northern New Zealand" and Williams⁶, Cotton⁷, and Ross and Smith⁸ have drawn similar conclusions. Acidic volcanic rocks, similar to those at Lake Toba, but on a reduced scale, occur widely in Sumatra, and in many other parts of the circum-Pacific region⁸. They seem to be an integral part of the calc-alkaline association of volcanic rocks associated with dipping seismic zones and deep trenches, although they are restricted to areas where crustal thickness exceeds about 25 km.

Is Lake Toba a potential source region for tektites? Volcanic theories for the origin of tektites have long been in disrepute⁹, principally because the observed distribution is too extensive (up to 4 per cent of the Earth's surface if the microtektite occurrences¹⁰ are included in the strewnfield) to be accounted for by eruptions from volcanoes. Thus Lake Toba would need to be an impact feature, because only meteorite, cometary or asteroidal impacts provide sufficient energy to account for the vast south-east Asia strewnfield. Lake Toba does not seem a particularly suitable candidate for an impact site. It is not circular, but elongated. The welded tuffs surrounding Toba are similar to other areas of undoubted volcanic origin in the circum-Pacific orogenic regions, and differ only in extent.

A decisive test is available from a comparison of the chemical composition of the welded tuffs with that of australites. The available data are collected in Table 1. The Toba analyses are old but probably reliable, and they agree with analyses of other welded tuffs. The central North Island ignimbrites generally have larger amounts of silica, but have similar contents of diagnostic elements such as magnesium¹¹, and individual matching analyses can be found¹².

An average of twelve australite analyses^{13,14} from localities extending from Charlotte Waters, NT, to Florieton, SA, along the line established by McColl and Williams² is given in column 5, Table 1. If the lake is the tektite source the compositions of the tuff and the tektites should be identical. It is clear from a study of the data in Table 1 that they are not. Particularly critical elements in this comparison are magnesium,

Table 1. COMPARISON OF CHEMICAL COMPOSITION OF WELDED TUFF FROM LAKE TOBA, SUMATRA (1-4), WITH THAT OF AUSTRALITES (5-9)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
SiO ₂	69.91	70.87	70.65	71.25	74.10	70.61	70.63	71.19	69.79
Al ₂ O ₃	16.00	15.05	17.32	14.21	10.90	13.50	13.07	13.28	13.06
FeO	3.35	2.55	4.36	1.19	4.30	4.77	4.95	4.95	5.48
MgO	0.94	0.76	0.39	0.89	1.92	2.31	2.48	2.40	2.89
CaO	2.88	2.28	2.28	2.72	4.00	3.41	3.53	2.95	3.64
Na ₂ O	3.32	3.41	2.85	3.11	1.20	1.52	1.51	1.51	1.33
K ₂ O	3.25	4.75	2.41	6.74	2.21	2.46	2.55	2.54	2.30
TiO ₂	0.33	0.37	—	—	0.65	0.75	0.78	0.76	0.77
	99.98	100.04	100.26	100.11	99.28	99.37	99.55	99.62	99.34

- (1) Welded rhyolite tuff, Lai Renoen, Toba Region⁴.
 - (2) Pumice from rhyolite tuff, Lau Matap, Kano Country, Toba region⁴.
 - (3) Welded rhyolite tuff, Sipak Peninsula, Lake Toba⁴.
 - (4) Welded rhyolite tuff, Central Batak, Toba region⁴.
 - (5) Average of ten australites from McColl-Williams Line. Anal. No. 8, 10, 11, 12, 13, 15, 16 (ref. 13) and Anal. No. 29, 31, 33 (ref. 14).
 - (6) Australite, Wiluna, WA Anal. 2 (ref. 13).
 - (7) Australite, Edjudina, WA Anal. 3 (ref. 13).
 - (8) Australite, Kalgoorlie, WA Anal. 6 (ref. 13).
 - (9) Australite, Lake Wilson, SA Anal. 26 (ref. 14).
- Data expressed as weight per cent oxides, on a volatile free basis. All iron expressed as FeO.

sodium and potassium. The australites contain more than twice as much magnesium and about half the sodium and potassium content of the welded tuffs. There is also a marked difference in the silica content. In order to ascertain whether these differences are dependent on silica content or sampling, I have made a comparison with other australites of similar silica content to the Lake Toba material. These comparisons are given in columns 6-9, Table 1, where typical australite analyses off the McColl-Williams line, but with silica contents of about 70 per cent, are listed. These analyses are again dissimilar to the Lake Toba material particularly for the critical elements magnesium, sodium and potassium.

These differences parallel those between the chemistry of tektites and terrestrial igneous rocks as has been repeatedly pointed out¹³⁻¹⁶. The chemical and isotopic composition is consistent with a parent material resembling terrestrial sedimentary rocks¹³⁻¹⁶.

The recent chemical, isotopic and age results from the lunar rocks¹⁷⁻¹⁹ show such a wide divergence from the tektite data as to make a lunar origin for tektites very improbable. It is thus necessary to locate another source of the south-east Asian strewnfield. The criteria for such a search have been discussed²⁰ and would be satisfied by cometary impact on shaly sandstones of Mesozoic age. There is, however, no *a priori* case for considering Lake Toba as a potential source area.

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Stratigraphy of the Beacon Supergroup between the Olympus and Boomerang Ranges, Victoria Land, Antarctica

In the summer of 1968–69 a geological expedition from Victoria University of Wellington examined the Beacon supergroup over an area of 3,500 km² between the Olympus and Boomerang ranges (Fig. 1). In this area the Beacon supergroup comprises as much as 2,300 m of flat-lying non-marine sediments from Devonian or older to Triassic in age. The season's work contributed to the regional geology in two ways: abundant Devonian freshwater fish remains were discovered and collected, thus extending the known geographic distribution and variety of the fossil assemblage; and a thin sequence of glacial beds of Late Palaeozoic age were found to be widespread throughout the area. Data from the measurement of thirty detailed stratigraphic sections have also led to considerable refinement of Beacon supergroup nomenclature (Table 1).

Harrington¹ subdivided the Beacon strata in southern Victoria Land into the Taylor and Victoria groups (Table 1). The sediments of the Taylor group are mostly texturally mature, exhibit only rare carbonaceous matter and contain Devonian fish and other fossils in the higher strata. By contrast, the Victoria group contains a heterogeneous assemblage of rocks which are texturally less mature and contain an abundance of carbonaceous material in which the *Glossopteris* flora may be present in the lower part and the *Dicroidium* flora in the upper part.

The Taylor group rests on the Kukri surface (Kukri peneplain²), a widespread surface cut in Pre-Cambrian–Lower Palaeozoic igneous and metamorphic rocks. The upper limit of the group is the Maya erosion surface¹. The lowest Taylor group formation in the area studied, the New Mountain sandstone, is a light-coloured cross-bedded quartzose sandstone, with local pebbly sandstone, cyclic siltstone and mudstone and breccio-conglomerate members in the lower part (Table 1). The formation decreases

in thickness northward from 220 m at Mt Handsley to 46 m near Mt Boreas (Fig. 1). The New Mountain sandstone is truncated by a widespread and previously unrecognized erosion surface, here named the Heimdall erosion surface. The overlying Altar Mountain formation, which is 162 m thick at the type section (McElroy at the Mar de Plata symposium, 1967), consists of a 30 m thick Odin Arkose member followed by a cyclic, extensively burrowed sequence of quartzose sandstone and maroon-green siltstones. The two succeeding formations comprise chiefly quartzose sandstone. The 385 m thick Arena sandstone is largely a buff-coloured slope-forming unit, in contrast to the overlying Beacon Heights orthoquartzite, which forms prominent white cliffs more than 300 m high. The Beacon Heights orthoquartzite passes gradationally up into a red and green siltstone sequence, the Aztec siltstone. The latter formation thins northward from 138 m in the Boomerang range to 40 m at Aztec Mountain. The abundant well preserved fish remains in this unit are discussed.

Apart from the Permian *Glossopteris* floras in the lower part of the Victoria group the only fossil material on record in southern Victoria Land for many years were Devonian freshwater fish remains found in erratics, near Granite Harbour³. In the summer of 1957–58, however, Warren², a geologist with the trans-Antarctic expedition, collected fragmentary fish remains (fossil descriptions by White⁴) from five localities in southern Victoria Land. One of the aims of the 1968–69 expedition was the collection of additional fossil fish material. Warren's locality in the northern Boomerang range (near Alligator Peak) was relocated and extensive collections were made. A considerable number of new localities were discovered and fossil material recovered from these included jaws, teeth, skulls, dermal plates and fin spines. These fossils represent the best preserved and most varied Devonian fish yet found in Antarctica. At one locality in the northern Boomerang range the fossil skeletal elements occur in such abundance as to form extensive bedding plane pavements. The largest and best preserved skeletal elements are usually concentrated in a few beds, each only a few inches in thickness. Smaller and much fragmented material is scattered by reworking through the greater part of the formation. The most rewarding localities were found in the southern part of the Olympus–Boomerang range area where the Aztec siltstone is thickest and siltstones are predominant over interbedded sandstones.

A Devonian age for the Aztec fish fauna is favoured by all authors. Woodward³ assigned it to the Upper Devonian, and White⁴ supported an Upper Middle Devonian age. Matz and Hayes⁵ regarded them simply as Devonian, and McElroy cites a Middle or preferably Upper Devonian age (Mar de Plata symposium). The only associated fossil material in the Aztec siltstone is a microflora of eight species (dated as lower Upper Devonian) from a horizon above the fish beds and near the top of the formation at Aztec Mountain⁶. The only other known Devonian fish material from Antarctica is a single plate of an arctolepid from the Horlick Formation of the Ohio Range⁷, which is Lower Devonian in age.

Harrington's¹ division of Beacon strata into Taylor and Victoria groups was based largely on the meagre data then available from Aztec Mountain, Maya Mountain and Mt Weller^{8,9}. He proposed the name Maya erosion surface for the disconformity between the Devonian Aztec siltstone and Per-

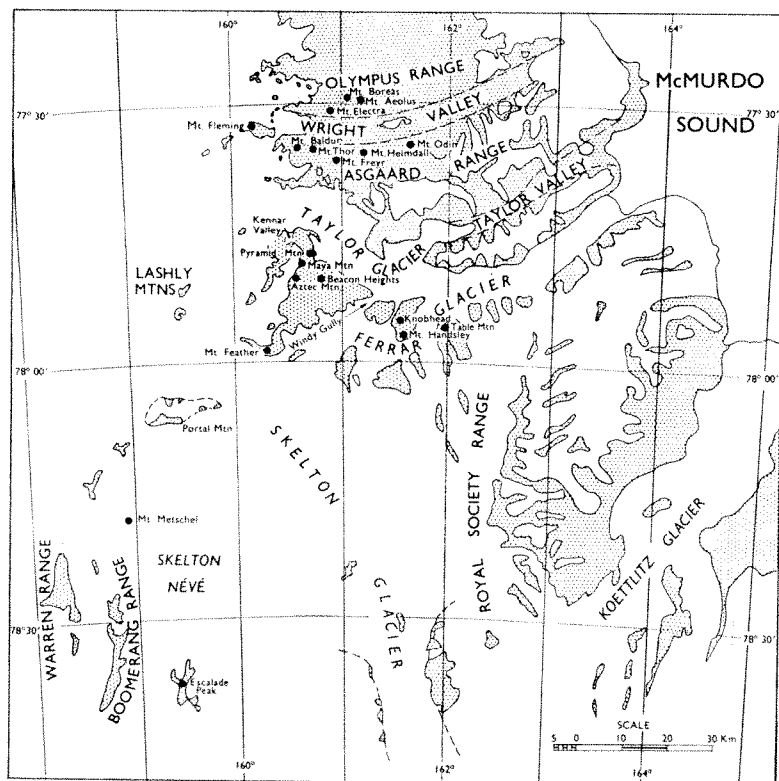


Fig. 1. Map of Olympus–Boomerang Ranges area Victoria Land.

Table 1. STRATIGRAPHIC NOMENCLATURE PROPOSED FOR THE BEACON SUPERGROUP OF THE OLYMPUS-BOOMERANG RANGES AREA

Age	From McElroy (Mar de Plata Symposium, 1967)			This report									
Triassic to Permian	Beacon group	Victoria sub-group	Lashly formation	Beacon supergroup	Victoria group	Lashly formation			Pyramid erosion surface				
Permian or ? Carboniferous			Feather conglomerate			Fleming formation (Nov.)				Maya erosion surface			
			Weller coal measures			Feather conglomerate							
Mid to Upper Devonian Lower to Mid Devonian			Not recognized			Weller coal measures					Metschel tillite (Nov.)		
		Aztec siltstone	Aztec siltstone										
		Beacon Heights orthoquartzite	Beacon Heights orthoquartzite										
		Arena sandstone	Arena sandstone										
		Altar Mountain formation	Altar Mountain formation										
		Devonian or Pre-Devonian	Taylor sub-group		New Mountain sandstone	Taylor group					New Mountain sandstone	Odin Arkose member	
Terra cotta silt-stone member												Boreas subgreywke. member	
Windy Gully sand-stone member													
Palaeozoic to Pre-Cambrian		Basement complex								Kukri surface			

mian Weller coal measures. As did earlier authors, Harrington drew attention to a glacial strata in the Devonian to Permian succession farther south in the Transantarctic Mountains and ascribed their absence in the Taylor Glacier area to non-deposition of glacial sediments on the glacially cut Maya erosion surface. Harrington correlated the disconformity at the base of glacial sediments of southern Transantarctic mountain localities¹⁰ with the disconformity (his Maya erosion surface) between the Aztec siltstone and Weller coal measures in the Aztec Mountain area.

As a result of the 1968-69 expedition it is now known that glacial beds are present in the Aztec and Maya Mountains sections and other localities in the Olympus-Boomerang ranges. The glacial beds (for which the name Metschel tillite is proposed) are disconformable on Aztec siltstone. Widespread erosion before the deposition of the Weller coal measures has removed the greater part or all of the glacial beds in many localities, destroyed the erosion surface at their base and cut down into the underlying Aztec siltstone. It was this upper disconformity that Harrington believed to be of glacial origin and for which he proposed the name the Maya erosion surface. We believe that the term Maya erosion surface is better reserved for the disconformity at the base of the glacial beds in the area of the Aztec-Maya Mountains. For the disconformity at the base of the Weller coal measures the name Pyramid erosion surface is proposed.

The lower boundary of the Victoria group is placed at the Aztec siltstone-Metschel tillite contact (= Maya erosion surface) where this exists, or the Aztec siltstone-Weller coal measures contact (= Pyramid erosion surface) where the Metschel tillite and Maya erosion surface have been eroded.

The name Metschel tillite is proposed for the glacial sequence of tillite, varvoid siltstone and associated stratified sediments. The formation is 27 m thick at Mt Metschel but reaches a maximum thickness of 38 m in the Boomerang range further to the south (Fig. 1). The tillite is usually massive with a grey-green sandy matrix in which scattered granitic, gneissic and metasedimentary clasts up to 1.5 m across occur. Large scale slumps are common in the stratified beds. The lower contact of the formation, the Maya erosion surface, is clearly disconformable to the north. At Aztec Mountain the surface bears a NW-SE oriented pattern of smoothly rounded elongate groove and ridge structures, possibly of glacial origin. To the

south, the Metschel tillite-Aztec siltstone contact is not as clear and requires more detailed investigation.

The Weller coal measures, which overlie the Pyramid erosion surface, consist of 170 m of cross-bedded carbonaceous sandstone and siltstone, with minor conglomerate lenses. Individual seams of bituminous to anthracite grade coal range up to a metre thick. The lower strata tend to be arkosic and include a basal conglomerate up to 15 cm thick, with clasts petrographically similar to those in the Metschel tillite, even where the tillite has been completely eroded. Leaves of *Glossopteris* and *Gangamopteris* indicate a Permian age for this formation.

McElroy (Mar de Plata symposium) proposed the name Feather conglomerate for 215 m of conglomerate and sandstone that overlie Weller coal measures at Mt Feather. We examined this formation at Mt Fleming and Portal Mountains and restrict the name to the lower conglomeratic beds. Thus the redefined Feather conglomerate is approximately equivalent to the unnamed 160 m thick middle Permian unit of Pinet, Matz and Hayes¹¹.

The name Fleming formation is proposed for 45 m of coarse, cross-bedded orthoquartzite and interbedded khaki to green siltstone that conformably overlies the redefined Feather formation at Mt Fleming. The Fleming formation is believed to be equivalent to the unnamed upper Permian unit of Pinet, Matz and Hayes¹¹.

The Lashly formation, youngest unit of the Victoria group, consists of 300 m of sandstone, shale and carbonaceous bands at the type section on Mt Feather (McElroy, Mar de Plata symposium). Similar strata were found near the summits of Mt Fleming and Portal Mountain. Plant microfossils collected from this formation at Mt Feather are probably late Middle Triassic⁶.

This subdivision of the Beacon supergroup into Taylor and Victoria groups has been firmly established. The nature of their contact is better understood following the discovery of Metschel tillite and the Maya and Pyramid erosion surfaces in the Aztec Mountain area. The Beacon section in the area studied is therefore more complete than previously thought and more akin to Devonian-Triassic successions farther south in the Transantarctic Mountains^{10,12}. Four major erosion surfaces, two previously unrecognized, are now known to exist in Beacon supergroup rocks between the Olympus and Boomerang ranges. Furthermore, the Aztec siltstone and its excellently preserved Devonian fish are now known to be distributed throughout the western part of this area. The formation

has yet to be reported beyond this area. Fossil fish collected during the 1968–69 field season have been sent to Dr A. Ritchie of the Australian Museum, Sydney^{13,14}. His comparison of Antarctic material with Devonian freshwater fish of Australia should lead to greater understanding of the similarities and differences between the faunas of the two continents and a refinement in the known geographic distribution of taxa in this fossil group.

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Some Non-linear Phenomena of Capillary Wave Propagation

STUDY of the interaction of nonlinear waves on a liquid surface is of considerable interest¹ because it may help to explain a great number of phenomena in wave dynamics of the sea. It is also relevant to capillary waves and the modelling of the nonlinear wave processes for dispersive media, which is of interest in nonlinear optics and electro-dynamics. The study of nonlinear capillary waves is also important for capillary hydrodynamics.

This report deals with some experiments on the propagation of nonlinear capillary progressive waves. The theory of nonlinear progressive capillary waves has been developed in a number of papers^{2–5}, but relatively little experimental work has been done on this subject^{6–7}. We have observed the second and higher harmonics which were generated during the propagation of capillary waves on a water surface and have noted the change in the form of oscillation in these waves.

We used a very simple device for the experiment. The almost plane capillary waves were generated by a light aluminium stick attached to a sound dynamic diffusor. The width of the light aluminium stick is approximately 10λ (for the low frequencies). The measurements were carried out at no more than $15\text{--}20\lambda$ from the aluminium stick and the diffraction effects were negligible. The homogeneity of the capillary wave field was controlled by means of stroboscopic light.

The measurements were carried out with technical water, which is a weak electrolyte. The device used as a receiver for the capillary waves consisted of a thin steel wire touching the surface of the water, which acted as the first electrode, and a copper plate as the other electrode plunged into the water underneath the wire. The distance between the copper electrode and the wire was 1.5 to 2 cm. A constant polarization voltage (10 V) was applied between the wire and the plate. In the propagating capillary wave the wire, which was 0.5–0.7 mm diameter, was periodically immersed in the water. The resistance between electrodes changed and as a result an alternating voltage emerged. The resistance of the waterlayer between the end of the wire and the other electrode was $\geq 20\text{ k}\Omega$. Thus the current between electrodes was less than 0.5 mA. The dependence of the probe current on the plunging depth was measured (static method). The current depended linearly on the depth, starting at $0.1 + 0.2\text{ mm}$. By means of the selective amplifier it was possible to carry out a harmonic analysis of the wave, to single out the second, third and higher harmonics successively. The amplifier had a broad frequency band so it was possible to observe the oscillating form of the surface.

The measurements were carried out at frequencies of 40–300 Hz and for amplitudes $10^{-1}\text{--}10^{-2}\text{ cm}$. The non-dimensional number $M = V/\lambda = 2\pi ac$ was of the order of 1. Here v is the oscillating velocity, c_1 is the phase velocity of the capillary waves, a is the displacement amplitude and λ is the capillary wavelength. The dispersive number for capillary waves is given by $D = (C_2 - C_1)/C_1 = 0.26$, where C_2 is the phase velocity for the wave with double the frequency. Our measurements therefore applied to the range $M \gtrsim D$ and dispersion could not effectively prevent the developing of nonlinear effects.

Fig. 1 shows the oscillogram of the second harmonic amplitude (logarithmic scale) and its dependence on the distance from the wave source; the frequency of the source was 200 Hz. The sharp upper lines are the distance marks at an interval of 1.5 cm. There are oscillations of the second harmonic amplitude caused by the differences between the phase velocities of the first and second harmonics. This is typical of nonlinear waves in the dispersive medium. Such nonsynchronous effects are well known in nonlinear acoustics (see for instance ref. 8) and in nonlinear optics⁹. It follows from the theory that the oscillation period Δ_{2f} is given by $\Delta_{2f} = 2\pi(2k_1 - k_2)^{-1}$, where k_1 and k_2 are the wave numbers for the first and second harmonics respectively. For capillary waves with their dispersive law we have $\Delta_{2f}/\lambda = 2.42$. Table 1 shows the values of Δ_{2f} for different frequencies very close to the experimental values. The experimental values λ , c and Δ_{2f} were obtained with our experimental set up and the measurements of λ and c were carried out with a phase interferometer on

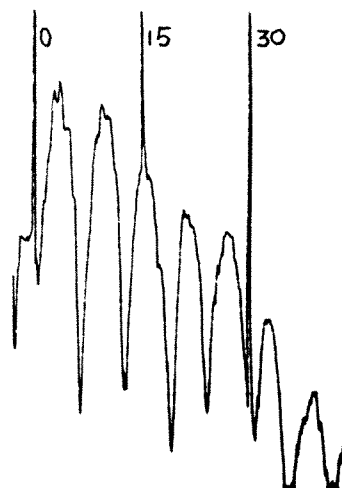


Fig. 1.

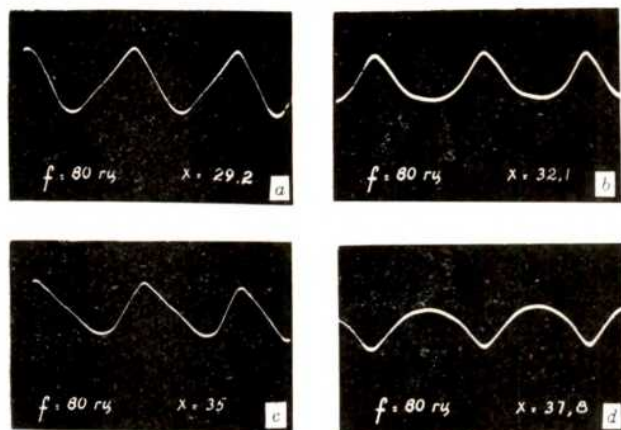


Fig. 2.

the Lissajous figures with an accuracy of 2–3 per cent. We deduce that the weak current between electrodes has no noticeable influence on σ . This phase interferometer proved to be very dependable. With the measured value of λ and c , σ was calculated. The difference between experimental and theoretical values of σ is small and on the low frequencies no more than 5 per cent. As can be seen from Table 1 the difference between theoretical and experimental values of Δ_{2f} is small and even on the high frequencies the error in Δ_{2f} is only ~ 10 per cent.

Table 1. THEORETICAL AND EXPERIMENTAL RESULTS OF THE VELOCITY C_1 , WAVELENGTH λ AND THE OSCILLATORY PERIOD Δ_{2f} OF THE SECOND HARMONIC

Frequency (Hz)	C_1 cm/s	Theory λ cm	Theory K cm ⁻¹	Δ_{2f} cm	Experiment λ cm	Experiment Δ_{2f} cm
40*	26.5	0.66	9.5	—	0.75	—
60*	30.2	0.50	12.5	1.23	0.53	1.1
80	33.5	0.41	15.2	1.00	0.41	1.0
120	38.0	0.32	19.9	0.78	0.30	0.75
160	41.9	0.26	24.1	—	0.26	—
240	48.2	0.20	31.5	—	—	—
335	54.3	0.16	38.5	0.39	0.14	0.35
670	68.5	0.10	61.7	—	0.09	—

* At the low frequencies the theoretical values c_1 , λ , k , were calculated with formulae for the capillary wave. The capillary waves occur when $K > (g/\sigma)\lambda$ where K is the wave number, g is the Earth's acceleration, σ is the density and σ is the surface tension coefficient. For water $(g/\sigma)^{1/2} = 3.65$ and at frequencies as low as 80 Hz the gravitation force is important.

The nonlinear distortion of the capillary wave is rather strong. For instance, at 80 Hz ($\lambda = 0.41$ cm) and for $a \approx 10^{-2}$ cm at the first maximum (a is the so called mathematical wave amplitude equal to the value half way between maximum and minimum amplitudes) the amplitude of the second harmonic was as large as 13–15 per cent as that of the first one.

We were unable to observe the stationary form of the wave profile which was predicted theoretically⁵. At every point on the surface the profile was stationary in the course of time; however, at different intervals from the source it varied periodically with the distance from the source. Fig. 2 shows the oscillograms of the wave profile at $a = 8.7 \times 10^{-2}$ cm and a frequency of 80 Hz. In Fig. 2a we see the profile form which is almost a back to front saw-tooth wave (the foremost time front is shallow but the back front is steep). As the distance becomes greater the form gets transformed and becomes the counter-form to that in the theory⁵ (see Fig. 2b). The further displacement of the receiver leads to a "shock" (saw-tooth) capillary wave (Fig. 2c) with a steep front, and then to the form predicted in ref. 5 (Fig. 2d). The latter form is in good agreement with theoretical predictions⁵ if we take into account the values of a/λ . Displacing the receiver further again leads to the counter form saw-tooth wave (Fig. 2a)—"shock"—and to the form predicted in ref. 5. The period of such transformations is approximately 3λ . These experiments were carried out at $M = 1.3 > D$ and in these conditions, even in the case of a dispersive medium, the form of the saw-

tooth wave appeared. Because of dispersion this wave is transformed quickly to other profiles.

It is difficult to carry out similar experiments for electromagnetic waves in the optical part of the spectra. We may expect, however, that for optical waves a saw-tooth profile will be formed if the non-dimensional numbers M and D are related roughly as in our experiments with capillary waves.

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Orientation of Vacuum Condensed Overgrowths through Amorphous Layers

A VERY striking phenomenon regarding the interaction between substrate and overgrowth atoms during vacuum deposition was recently reported by Distler *et al.*^{1,2}. This phenomenon is the oriented nucleation of gold on NaCl substrates precoated with amorphous carbon layers below a critical thickness. Distler *et al.* interpreted their results in terms of long-range active centres^{3,4} which are present on the substrate surfaces in densities corresponding to that of gold nuclei and concluded that "oriented crystallization of gold through carbon films indicates that neither the microgeometry of the surface nor the fit in lattice parameters of NaCl and gold are of decisive importance for epitaxy"².

In view of the great significance of these observations, Chopra⁵ carried out careful and systematic experiments in an attempt to confirm them. His results, however, did not agree with the observations of Distler *et al.* Barna *et al.*⁶ studied the growth of ZnS and PbS on NaCl substrates that were precoated with SiO or C layers of various thicknesses. Although these are not strictly comparable with the Au/C/NaCl system, it is worth mentioning here that PbS grew only partially oriented on air-cleaved NaCl precoated with ~ 130 Å of C.

I have carried out some experiments which confirm the observations both of Distler *et al.* and of Chopra. Various corresponding portions of air cleaved and vacuum cleaved ($\sim 10^{-8}$ Torr) NaCl and KCl single crystals at $\sim 200^\circ$ C were simultaneously coated with carbon layers of various thicknesses in an ultra high vacuum system (10^{-7} to 10^{-6} Torr during carbon evaporation). Gold was then deposited at a rate of ~ 2 Å/s on these substrates at 340° C while certain portions of bare surface and of carbon coated regions were screened. Thin (~ 20 Å) as well as thick (~ 100 Å) gold films were deposited on every region of the substrates. A detailed account of the results will be published at a later stage and only the most important aspects will be mentioned here.

Fig. 1 shows the electron micrograph and diffraction patterns of a thick (~ 1000 Å) gold film that was deposited on the region of a vacuum cleaved NaCl crystal which carried the boundary between a 105 Å (left) and a 150 Å

(right) carbon coating. It is to be noted that polycrystalline gold film on the thicker carbon layer displays agglomerated polycrystalline grains whereas the perfectly single crystalline gold film on the thinner carbon layer is much more uniform and continuous with many microtwins. Similar results were obtained on the vacuum cleaved KCl crystal. The variation of surface mobility of gold nuclei on successively thicker carbon layers seems to be an important factor in determining the structure. The initially nucleated gold deposit on the left hand region of vacuum cleaved NaCl, shown in Fig. 1, revealed completely randomly oriented gold nuclei, as shown in the diffraction pattern in Fig. 2. It is only during the further growth and coalescence of nuclei that a perfectly oriented gold film results. The thicknesses of the carbon layers were determined by means of an oscillating quartz crystal microbalance. Fig. 3 shows an electron micrograph of a portion of the 105 Å carbon layer screened during gold deposition. The diffraction pattern showed that this carbon film was amorphous and from Fig. 3 it is clear that the film was without large discontinuities or holes. These results therefore confirm the controversial observations of Distler *et al.* It is also noted that the transition from single crystalline to polycrystalline gold deposits occurs over a rather small range of carbon layer thicknesses which makes the critical carbon thickness a very sensitive parameter for quantitative experiments on this phenomenon.

The gold films that were grown on the carbon coated portions of air cleaved NaCl and KCl crystals were all polycrystalline. Not even on 45 Å carbon layers could thick single crystalline gold films be obtained, although

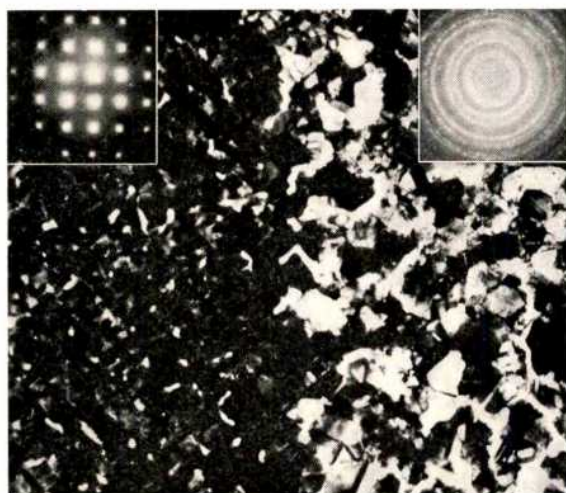


Fig. 1. Transmission electron micrograph and diffraction patterns of ~1000 Å gold film on the region of a vacuum cleaved NaCl crystal precoated with relatively thin (left) and slightly thicker (right) carbon layers. $\times 16,000$.



Fig. 2. Transmission electron diffraction pattern of initially nucleated gold deposit (~20 Å) on the left hand region of vacuum cleaved NaCl shown in Fig. 1.



Fig. 3. Electron micrograph of a corresponding portion of the carbon layer on vacuum cleaved NaCl to the left of Fig. 1. $\times 24,000$.

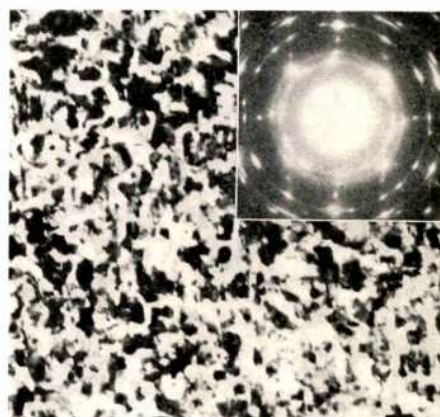


Fig. 4. Transmission electron micrograph and diffraction pattern of ~1000 Å gold film on air cleaved NaCl precoated with a carbon layer corresponding to that on the left of Fig. 1.

strong preference for the (001) parallel orientation occurred as may be seen from Fig. 4. In fact, the electron micrographs of the gold films revealed large patches of (001) orientation mixed with equally large areas of polycrystalline orientations. Above 150 Å carbon layer thickness this patchlike nature disappears and the thick gold film becomes completely polycrystalline. The latter results therefore confirm Chopra's observations. It is believed that he must have used air cleaved substrates because he quoted them merely as having been "freshly cleaved" and not as vacuum cleaved. It is possible that the air cleaved surfaces are either already covered with a thin amorphous water layer or that the surface layer of the crystal is altered in such a way that it does not contribute to long range forces to the same extent as does the parent surface layer of a vacuum cleaved crystal. This could possibly be the case, for example, when the chlorine ions of the parent surface layer are replaced by hydroxide ions, leading to a relatively smoother surface potential⁷.

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High Energy Photoelectron Spectroscopic Study of Carbon Fibre Surfaces

KNOWLEDGE of the internal structure of carbon fibres, which are derived from polyacrylonitrile and rayon by carbonization in special conditions¹⁻³, has increased significantly in the past few years. Investigations using small-angle and conventional X-ray diffraction⁴⁻⁹, electron diffraction^{6,10,11}, intercalation with caesium¹², helium density determinations⁶, polarized light microscopy¹¹, and high-resolution electron microscopy^{6,13}, have established that the carbon layers form long (greater than 100 nm) ribbons with preferred orientation of the layers parallel to the fibre axis. A number of these parallel ribbons form a so-called microfibril⁶, the average thickness (or stack size) of which, in fibres that have been heat treated to 1,300 K or beyond, is in the range 3-10 nm. The microfibrils are believed to be wrinkled and bent^{6,9} and they may even be twisted to form rather inaccessible voids. Within the layers, the C-C bond distance⁹ is 1.416 ± 0.007 Å, and for this, and other reasons⁶, the bonding is believed to involve only sp^2 hybridized carbon atoms. There is no evidence of cross-linking between the layers (which contain a few per cent of structural defects⁹) perpendicular to the axis of the microfibrils.

The commercial exploitation of carbon fibres hinges on the discovery of effective methods of increasing the interfacial shear strength between the cylindrical fibre surface and the compatible material with which they are formed into composites. Knowledge of the atomic structure of the exterior surfaces of carbon fibres is therefore essential, and the surface structures have recently been studied by physical adsorption and chemisorption of gases¹⁴. Reflexion electron diffraction¹¹ has also been used, for the structures thus revealed refer to depths of less than 100 nm from the surface. We describe here a new approach to the direct study of surface structures: the use of high energy photoelectron spectroscopy¹⁵

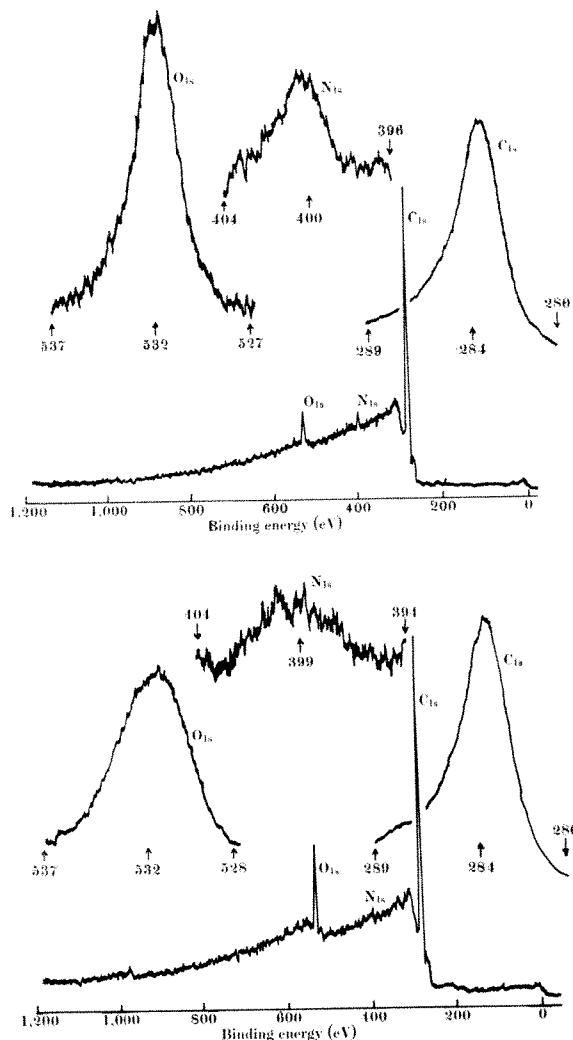


Fig. 2. High energy photoelectron spectra of nitrogen 1s, oxygen 1s, and carbon 1s electrons in Courteille carbon fibre, baked at approximately 1,300 K in nitrogen (type b or type II)—top spectra; and spectra of the same fibre subjected to oxidation (type c)—bottom spectra. There is an appreciable increase in oxygen bound to the surface following oxidation.

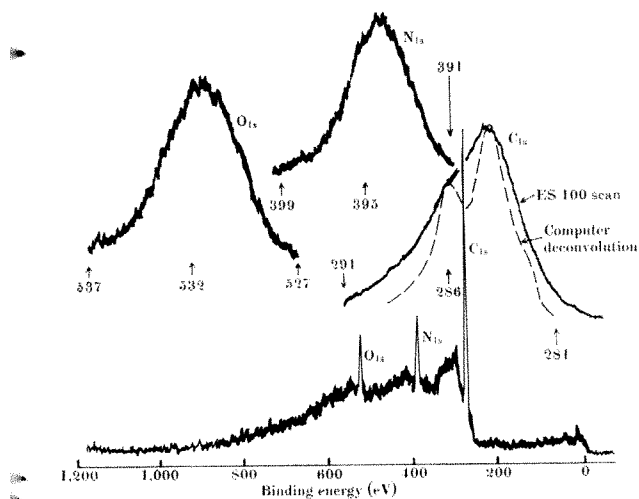


Fig. 1. High energy photoelectron spectra of nitrogen 1s, oxygen 1s, and carbon 1s electrons in oxidized Courteille carbon fibre (type a). The shoulder on the higher energy side of the carbon 1s peak indicates two distinct kinds of carbon atoms. From the width of the oxygen 1s and nitrogen 1s peaks there are at least two kinds of oxygen and nitrogen atoms present.

which has already proved valuable in a number of disparate fields¹⁵⁻¹⁸. Its great advantage in the present context is that only a very thin surface layer—estimated to be approximately 10 to 15 nm—contributes to the spectra. The method entails direct measurement of the binding energies of electrons in the 1s levels of carbon, oxygen and nitrogen. It has already been demonstrated by Siegbahn¹⁵ and others¹⁶⁻¹⁹ that shifts in energies of the core electrons are sensitive to the chemical environment of the atoms.

The electron spectra were excited by characteristic aluminium K α radiation (1487 eV) and measured with the AEI Scientific Apparatus Ltd ES 100 spectrometer. The binding energies, E_b , have not been corrected for the work function of the spectrometer nor for charging effects wrought by the ionizing radiation. Five types of polyacrylonitrile-based fibres were examined: (a) oxidized Courteille, (b) Type II, a Courteille fibre baked at approximately 1,300 K in nitrogen; (c) the same type II but oxidized to improve the bonding of the surface to a matrix, (d) type I, a Courteille fibre baked at approximately 2,400 K in argon and (e) the same type I, but oxidized.

The relevant results are shown in Figs. 1-3. Several features need comment. In the first place, the technique clearly reveals the appreciable increase in oxygen bound to the surface after oxidation. From absolute counts

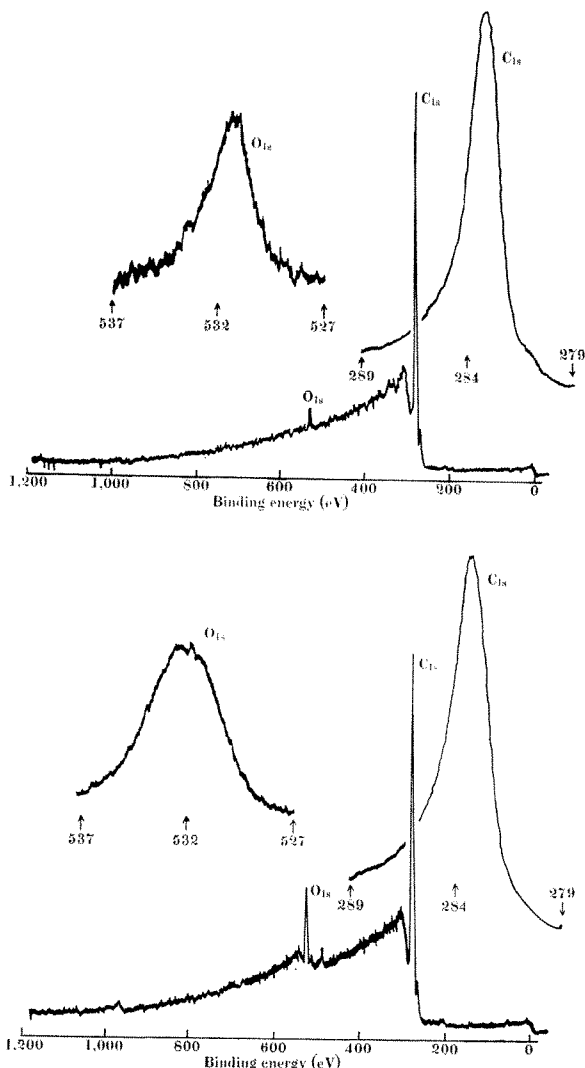


Fig. 3. High energy photoelectron spectra of nitrogen 1s, oxygen 1s and carbon 1s electrons in Courteille carbon fibre, baked at approximately 2400 K in argon (type d or type I)—top spectra; and spectra of the same fibre subjected to oxidation (type e)—bottom spectra.

recorded in other systems (unpublished work), it is estimated that there are five oxygen atoms per 100 carbon atoms in type (e). Second, from the expected half-width of the oxygen peaks (1.8 eV), it is certain that there are at least two kinds of oxygen in all of the oxidized fibres. By the same token, there are two distinct kinds of carbon atoms in sample (a). From the known shifts which have been observed by Siegbahn¹⁵, and ourselves (ref. 17 and unpublished work), the shoulder on the higher binding-energy side of the carbon 1s peak in Fig. 1 cannot be assigned to simple aliphatic carbonyls, aldehydes, carboxyl or amide groups, because the observed shift is too small. An aliphatic ether linkage may likewise be discounted because the shift is too large. (Further work, now in progress, on model aromatic compounds, will clarify the situation.) It should be noted that the profile of carbon 1s peaks in the heat-treated samples (b to e) bear no resemblance to the corresponding peak in diamond or to the carbon 1s peak in other materials, such as linear saturated hydrocarbons, where the bonding involves sp^3 hybridization. Moreover, the profile is closely similar to that obtained for graphite and vitreous carbon, which supports the suggestion⁶ that the structure of isotropic non-graphitizable carbons (vitreous or glassy carbons) might be interpreted in terms of a ribbon model, with suitable allowance for branching.

Another noteworthy feature is that almost all nitrogen atoms are driven off from the carbon surfaces by heating to 2,300 K. Spectra at high sensitivity recorded with the ES 100 spectrometer have revealed that fibres of type (b) nevertheless contain at least two kinds of nitrogen atoms, the total percentage of which, within the penetration depth, amounts to about 1 per cent.

It is clear that by utilizing higher sensitivity and if feasible, higher resolution, along with appropriate unfolding techniques, it should prove possible to identify precisely the nature of the surface bonding and to clarify the chemical structure of carbon fibres.

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BIOLOGICAL SCIENCES

Brain Fatty Acid Elongation and Multiple Sclerosis

ELONGATED fatty acids in the brain are of interest because they are thought to contribute to membrane stability and cohesiveness^{1,2}. Using a technique similar to that described by Aeberhard and Menkes³, the developmental pattern of fatty acid elongation was assessed in the rat brain⁴.

Mitochondria were isolated by discontinuous sucrose gradient centrifugation from rat brains 5, 10, 15 and 20 days of age and in adulthood. Duplicate samples of 1 mg mitochondrial protein from each age group were incubated for 20 min under nitrogen in a buffered medium NADPH, NADH, ATP, and acetyl- $1-^{14}C$ -CoA. Fatty acids were extracted, transmethylated, purified, and analysed by gas-liquid chromatography. Stream-splitting allowed capillary collection of eighteen fractions for radioactive counting of individual fatty acids. Fatty acids were definitely identified by combined gas-liquid chromatography with mass spectroscopy.

The results show that fatty acid elongation reaches its maximum rate during myelination. Interestingly, the pattern of fatty acid elongation changes with brain growth and development. In younger brains, the mitochondria synthesize primarily the longer chain fatty acids, particularly $C_{22:4}$ (39.3 per cent), while the older brains form primarily the shorter chain fatty acids, particularly $C_{18:0}$ (17.7 per cent) and $C_{20:1}$ (15.3 per cent).

Of possible relevance to these findings is the report of Gerstl and colleagues⁵ that the two prominent fatty acids deficient in (frozen) multiple sclerotic brains were $C_{20:1}$ from ethanolamine glycerophosphatide and $C_{24:1}$ from cerebroside. They conclude from their data that an inadequacy of the fatty-acid elongation process and a deficit of cerebrosides represent two of the early biochemical lesions in the multiple sclerotic white matter.

Our findings, however, of a distinctive developmental prominence of $C_{20:1}$ and $C_{22:4}$ would support the view that changes in early human brain growth and development, specifically the synthesis of $C_{20:1}$, influence the eventual predisposition and development of multiple sclerosis⁶. Suggested areas of fruitful research are the control mechanisms that regulate the synthesis of elongated fatty acids and the role these fatty acids (or their elongation products) play in membrane stability, as in model membrane systems.

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Localization of Encephalitogenic Basic Protein in the Intraperiod Line of Lamellar Myelin

IN the course of investigations on the immunogenic hypothesis of multiple sclerosis we have attempted the preparation of a "chemically pure myelin" and come to the conclusion that the giant molecular complex which composes the structure which the morphologist identifies as myelin has a certain integral molecular component which displays such a differentially high order of instability that this may be of great biological significance. A specific protein material is released rapidly *post mortem* from the intraperiod line of myelin and also from ultracentrifugally purified myelin *in vitro*, in conditions which make enzyme mediation improbable, and the myelin so obtained has a characteristic structure which we term "collapsed myelin". Little else besides this protein seems to be released; and this protein has been shown to be a single entity identical with the "basic encephalitogenic protein" of central nervous tissue¹⁻³.

The myelin was prepared from bovine cerebral white matter by an ultracentrifugation method modified from that of Autilio *et al.*⁴. The equilibrium density centrifugal distribution of water-washed, crude myelin product in preformed continuous sucrose density gradients varied according to the time that elapsed after the death of the animal before the excised white matter was thoroughly chilled to 4° C (K. M. J., J. P. D., S. R. A. and C. E. L., to be published). If this interval was no more than 20 min, the pattern was of a single thick band centred

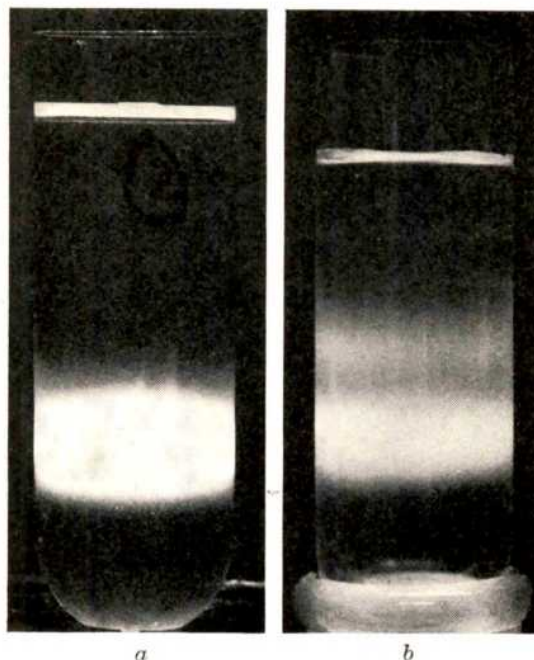


Fig. 1. *a*, Density gradient showing single band of myelin from bovine brain almost instantly chilled *post mortem*. *b*, The same showing four (the two uppermost very faint) bands of myelin from bovine brain collected in chilled conditions 50-60 min *post mortem*.

at density 1.084 g cm⁻³ (Fig. 1*a*) and this product was a well preserved myelin of normal electron microscopic morphology with a repeating lamellar period of 110-120 Å (Fig. 2*a*). As the length of time *post mortem* to chilling was extended up to about 2 h, further bands appeared (Fig. 1*b*), the chief of which was centred at density 1.063 g cm⁻³ and which showed, in addition to morphologically normal myelin, a collapsed lamellar structure of 100-105 Å periodicity in which the normal faint intraperiod line was replaced by a narrow line of similar electron density to the dense line (Fig. 2*b*). A possibly identical structure has in fact been depicted in the literature⁵, as an incidental observation, and Finean⁶ has related physicochemical changes of this character to dehydration in the instance of intact peripheral nerve.

The reduced flotation density associated with these collapsed myelin structures, as well as the chemical analysis data of the myelin fractions seem to correspond to the loss of predominantly proteinaceous material from the intraperiod lines, as shown in Table 1. The corresponding data on lipid analyses will be published elsewhere.

Table 1. CHARACTERISTICS OF FRACTIONS

Ultracentrifugation layer	Mean density (g cm ⁻³)	Protein content* (mean ± s.d.)
"Collapsed" myelin	1.063	23.75 ± 0.33
Intact lamellar myelin	1.084	34.38 ± 0.85

(* Expressed as weight-equivalent of bovine serum albumin per cent.)

Incubation of initially normal myelin (which has been shown to be enzyme-free) in saline-bicarbonate buffer (pH 7.4) at 37° C for periods up to 7 days resulted in no morphological or chemical changes. Incubation in lactate buffer at pH 3.5, or in 0.1 M HCl, however, caused the almost instantaneous appearance of collapsed myelin structures, and protein appeared simultaneously in the incubation medium. Electrophoresis of the latter in 15 per cent polyacrylamide gels at pH 4.0 revealed only one protein band and this was of high mobility, as a cation. Chromatography on carboxymethyl-cellulose with a mixed H⁺/Na⁺ gradient resolved all of the material absorbing at 280 nm in a single, symmetrical peak. The amino-acid compositions of the crude incubation supernatants and of the protein therefrom, after chromato-

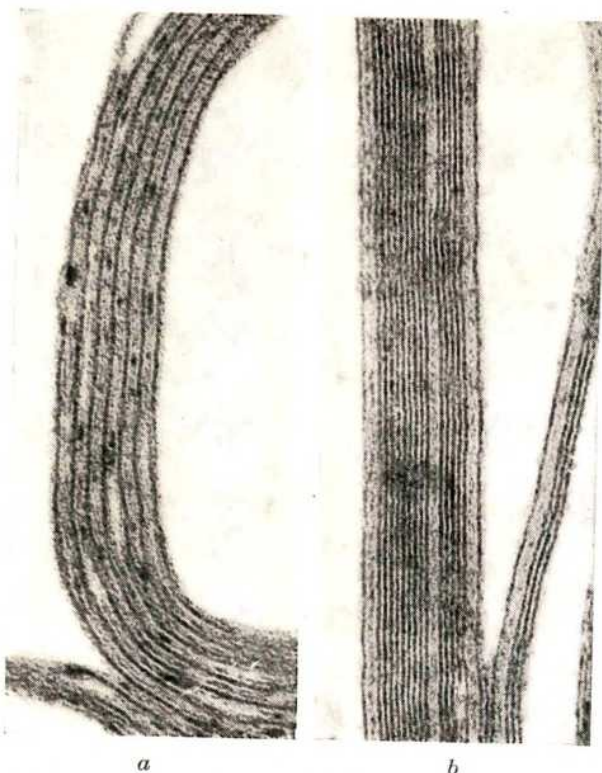


Fig. 2. *a*, Electron micrograph ($\times 152,000$) from "single-band myelin" as in Fig. 1*a*, showing intact intraperiod line. *b*, Electron micrograph ($\times 152,000$) from the third band of the 4-band myelin of Fig. 1*b* (the upper of the two obvious bands, ignoring the two topmost very faint bands).

graphy and ultrafiltration, were closely similar—except for the presence of sphingosine, ethanolamine and excess serine in the 0.1 M HCl supernatants (derived from hydrolysis of cerebroside and of ethanolamine and serine phosphatides). The supernatants and the purified protein were highly encephalitogenic in guinea-pigs at doses of 1 μ g (unpublished results of J. T. D., K. M. J., S. R. A. and C. E. L.). In all these examinations, the protein released from myelin was identical with basic encephalitogenic protein prepared from bovine cerebral white matter by standard defatting and acid extraction procedures for this purpose.

Analysis has shown that the released protein has the amino-acid composition: Asp₁₂, Thr₈, Ser₁₇, Glu₁₄, Pro₁₃, Gly₂₃, Ala₁₅, Val₅, Met₂, Ile₄, Leu₁₀, Tyr₄, Phe₇, (NH₂)₉, Lys₁₆, His₉, Trp₁, Arg₁₅; and a molecular weight of about 19,200 on the basis of the amino-acid analyses. The molecular weight, the tyrosine and tryptophan contents, and the ultraviolet absorption data⁷ are consistent with one another. The protein is therefore similar to that prepared from bovine spinal cord by Eylar *et al.*⁸ and by Hashim and Eylar⁹.

These observations do not, of course, imply restriction of the basic protein to the intraperiod line of the lamellar myelin (although such restriction seems probable on other grounds) but they indicate that the only protein present at this site is the basic protein. It may be significant that parallel studies in our tissue culture laboratory (C. E. L. and M. Jennings, to be published) suggest that demyelinating anti-basic-protein antibody reacts much more rapidly than other anti-brain antibodies in these cultures. The ease with which encephalitogenic protein may be released from the myelin lamellar intraperiod lines (and, *ipso facto*, into the extracellular space of nervous tissue) warrants serious consideration in the discussion of the pathogenesis of spontaneous autoimmune demyelinating disease, and we hope to delineate

the minimum deviations from physiological conditions required to release basic protein from myelin *in vitro*.

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Asparaginyl-tRNA and Resistance of Murine Leukaemias to L-Asparaginase

INTEREST in the enzyme L-asparaginase has been stimulated by the observation that it inhibits the growth of experimental and human tumours¹⁻³. Tumours sensitive to L-asparaginase require exogenous L-asparagine whereas tumours not sensitive synthesize L-asparagine from L-aspartic acid and L-glutamine. Moreover, if L-asparaginase-sensitive tumours are made resistant using suboptimal doses of the enzyme, the resistant variant possesses L-asparagine synthetase and no longer requires exogenous L-asparagine for growth^{3,4}. The critical questions now concern the regulation of L-asparagine synthetase activity.

The control of amino-acid biosynthesis seems to involve specific aminoacyl-tRNAs by direct interaction of the aminoacyl-tRNAs with a biosynthetic enzyme⁵ or by acting as repressor or co-repressor of the operon for enzymes involved in biosynthesis of the amino-acid⁶⁻⁹. Thus the amount of the L-asparagine biosynthetic enzyme, L-asparagine synthetase, may be regulated by the total amount of asparaginyl-tRNA or by the presence of a new species of asparaginyl-tRNA which acts as a repressor or a direct inhibitor. We describe here the asparaginyl-tRNA found in a leukaemia (L5178Y) lacking L-asparagine synthetase activity ("repressed") and sensitive to L-asparaginase, and in a leukaemia (L1210) possessing high L-asparagine synthetase ("derepressed") and therefore resistant to L-asparaginase³.

Murine leukaemia L1210 and L5178Y were carried in CD₂F₁ mice and were transplanted into mice of the same strains by inoculation of a dilution of the ascitic fluid. Mice were killed; the ascitic fluid was removed and centrifuged; and the cells obtained were frozen at -170°C before use.

Transfer RNA was isolated after deproteinization with phenol¹⁰, followed by chloroform extraction. The tRNA of the aqueous phase was precipitated with ethanol after each deproteinization. The precipitate was extracted with 1 M NaCl, and the supernatant containing tRNA was precipitated with ethanol. The precipitate was dissolved

in 0.1 M Tris-HCl buffer (pH 9.2) and deacylated. The sample was then neutralized and treated with deoxyribonuclease (Worthington, electrophoretically pure). Deoxyribonuclease was removed by phenol extraction and the tRNA precipitated, dried, and stored in a liquid nitrogen refrigerator at -170°C until use.

To prepare asparaginyl-tRNA synthetase, cells were homogenized in one volume of 0.1 M Tris-HCl buffer (pH 7.3) and centrifuged at $100,000g$ for 1 h. The supernatant of this step was used for the source of enzyme after addition of dithiothreitol and glycerol to final concentrations of 6 mM and 50 per cent, respectively. The samples were stored at -30°C until use. Protein was determined by the method of Lowry *et al.*¹¹.

The kinetics of asparaginyl-tRNA synthesis were determined in 0.1 ml. reaction mixtures containing 0.05 M KCl, 0.02 M magnesium acetate, 0.005 M ATP, 0.005 M CTP, 0.1 M Tris-HCl (pH 7.6), 0.001 M β -mercaptoethanol, 6.2×10^{-5} M L^{14}C -asparagine (specific activity 207 mCi/mmol, Amersham/Searle), 3×10^{-5} M each of the remaining unlabelled amino-acids except glutamine, glutamic acid and aspartic acid, 1.6 A_{260} units of tRNA and 8.0 μg of protein. L^3H -asparagine and L^{14}C -asparagine were purified by Dowex-1 ion exchange chromatography. Incubations were performed at 37°C and terminated with cold 10 per cent trichloroacetic acid (TCA). Precipitates were collected on 'Millipore' filters, dried, and the radioactivity was counted.

Acylation of tRNA before column chromatography was accomplished by increasing the reaction mixtures to 1.0 ml. and the tRNA and enzyme were proportionately increased. The tRNA was determined to be limiting by preliminary acylation studies in which concentrations of tRNA and enzyme were varied. Incubations were for 15 min at 37°C and terminated by phenol extraction. ^{14}C and ^3H -Asn-tRNA were separated from their respective labelled amino-acids by 'Sephadex G-25' chromatography. The Asn-tRNA was lyophilized, taken up in buffer, and applied to a 'Freon' column¹² for fractionation of isoaccepting species. Details of tRNA purification and acylation are described elsewhere (ref. 13 and unpublished work of R. C. G. and S. Pestka.) Conditions for conducting the 'Freon' column co-chromatography are described in the legend.

The total tRNA from these leukaemic cells varied from 6 to 25 A_{260} units/g of cells. There was no difference in the amount of tRNA obtained from L1210 and L5178Y cells. The amount of tRNA^{Asn} in L1210 and L5178Y cells was evaluated by comparing the amount of Asn-tRNA synthesized from $\text{tRNA}_{\text{L1210}}$ and $\text{tRNA}_{\text{L5178Y}}$ with L1210, L5178Y, and normal mouse liver (CD_2F_1) aminoacyl-tRNA synthetases. There was no difference in the rate or total amount of Asn-tRNA formed with any of the enzyme preparations when the same tRNA was used as amino-acid acceptor. The total Asn-tRNA synthesized was, however, approximately two-fold greater with $\text{tRNA}_{\text{L5178Y}}$ (19 pmoles/ A_{260} of tRNA) than with $\text{tRNA}_{\text{L1210}}$ (8 pmoles/ A_{260} of tRNA) with either L1210 or L5178Y aminoacyl-tRNA synthetase.

Fractionations of Asn-tRNA from L1210 and L5178Y by 'Freon' column co-chromatography are shown in Fig. 1. Three peaks of Asn-tRNA (isoaccepting species) were found with L1210 cells: a principal peak 1, an inter-

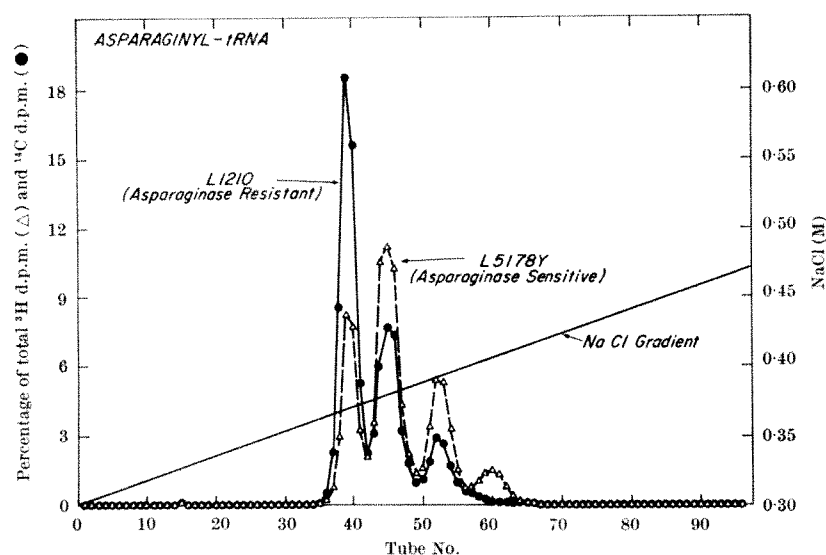


Fig. 1. Co-chromatography of ^3H -Asn-tRNA (90,581 d.p.m.) synthesized from L5178Y tRNA and L5178Y asparaginyl-tRNA synthetase and ^{14}C -Asn-tRNA (44,476 d.p.m.) synthesized from L1210 tRNA and L1210 asparaginyl-tRNA synthetase on a 'Freon' reverse phase partition column. The buffer was 0.01 M sodium acetate buffer (pH 4.5) containing 0.01 M MgCl_2 , 0.001 M EDTA, and a linear gradient of NaCl (0.30–0.65 M). The column was 1 cm \times 240 cm. Chromatography was performed at 20°C with a flow rate of 1.5 ml./min. Fractions (10 ml.) were collected and the Asn-tRNA was precipitated with 1.25 ml. of ice-cold 100 per cent TCA after the addition of 4 A_{260} units of carrier calf thymus DNA. The precipitates were collected on 'Millipore' filters, washed with 5 per cent TCA, dried by an infrared lamp, and counted in a liquid scintillation counter. Two hundred samples were collected and analysed, but only results for those containing the significant portion of the tRNA elution profile were plotted. The curves were normalized by determining the total ^3H and ^{14}C eluted from the column and expressing the ^3H and ^{14}C radioactivity of each tube as a per cent of the total ^3H and ^{14}C radioactivity respectively. These normalized values were plotted.

mediate peak 2, and a small peak 3. This pattern is identical to the Asn-tRNA profiles of normal human and mouse tissues which contain relatively high levels of asparagine synthetase ('derepressed') (our unpublished work). The Asn-tRNA profile of L5178Y differs, however, from L1210 (Fig. 1) and from normal mouse organs or human cells. Peak 1 is relatively less than peak 1 of L1210; peaks 2 and 3 are relatively greater; and an additional minor unique species (peak 4) was also fractionated. These findings were reproduced with the labelled amino-acids reversed.

To determine whether the differences obtained for Asn-tRNA with L5178Y were attributable to RNA itself or the asparagine-activating enzyme (asparaginyl-tRNA synthetase), L5178Y tRNA was acylated with asparagine-activating enzyme from L1210 and L1210 tRNA with enzyme obtained from L5178Y cells. The profiles were identical to those shown in Fig. 1, in which the autologous enzymes were used; the smaller peak 1, larger peaks 2 and 3, and peak 4 were found with L5178Y tRNA acylated with the L1210 enzyme. Thus the pattern obtained was not dependent on the asparagine activating enzyme, but was attributable to the tRNA itself.

The possibilities that peak 4 resulted from a partial degradation of a species of $\text{tRNA}_{\text{L5178Y}}$ during the isolation procedure, from a tRNA aggregate, or from a contaminating nuclease were ruled out by various experiments¹⁴. Furthermore, after isolation and deacylation of peak 4 the resulting product was shown to have an R_f identical to that of L-asparagine on paper chromatography in two solvent systems. The reproducibility of the differences in Asn-tRNA profiles between L1210 and L5178Y was tested by repeating tRNA isolation, acylation, and co-chromatography at three separate intervals on three different groups of animals. In each case results were identical to those presented in Fig. 1. We therefore conclude that the peaks of Asn-tRNA fractionated from these cells are isoaccepting species of tRNA^{Asn} and

differences in the peaks between cells "derepressed" and "repressed" for asparagine synthetase are true tRNA differences. The difference in tRNA profiles between L1210 and L5178Y cells is specific for L-asparagine, for a comparison of other aminoacyl-tRNA profiles has not revealed differences. These quantitative differences and in particular the presence of the unique small species of Asn-tRNA in the asparagine synthetase "repressed" cells (L5178Y) implicate Asn-tRNA in the regulation of asparagine synthetase.

We suggest that peak 4 of L5178Y may be a unique corepressor. In its absence asparagine synthetase is derepressed, resulting in resistance to the anti-leukaemic effects of L-asparaginase. Alternatively, the mechanism may be more complicated, involving differences between total Asn-tRNA levels of these cells as suggested in the control of the histidine operon by His-tRNA⁹.

Subsequent experiments are being performed to determine: (1) the Asn-tRNA profiles of other L-asparaginase sensitive and resistant tumours, (2) whether peak 4 Asn-tRNA interacts with asparagine synthetase, and (3) whether the addition of Asn-tRNA of L5178Y (particularly peak 4) will result in repression of asparagine synthetase and therefore sensitivity to therapy with L-asparaginase to derepressed leukaemic cells (L1210).

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Antineoplastic Activity of Highly Purified Bacterial Glutaminases

SEVERAL lines of evidence motivated the treatment of neoplasms by glutaminase to cause glutamine deprivation. Certain tumour cells grown in tissue culture require glutamine at a level which is tenfold or greater than that of any other amino-acid^{1,2}. Glutamine participates in a wide variety of metabolic reactions in mammalian cells³. It has been suggested that one of the important functions of glutamine in the metabolism of certain tumours may be as a direct precursor of glutamic acid, which can then furnish the carbon for the partial operation of the tricarboxylic acid cycle from α -ketoglutarate to oxaloacetate⁴. Compared with other tissues, certain tumour cells seem to operate at a marginal level of glutamine availability because of slow synthesis⁵ and rapid utilization⁴. The glutamine antagonists, azaserine and 6-diazo-5-oxonorleucine (DON) have been shown to possess moderate antineoplastic activity, which may be enhanced by L-asparaginase⁶⁻⁸. Greenberg *et al.*⁹ reported that a glutaminase-asparaginase preparation with a

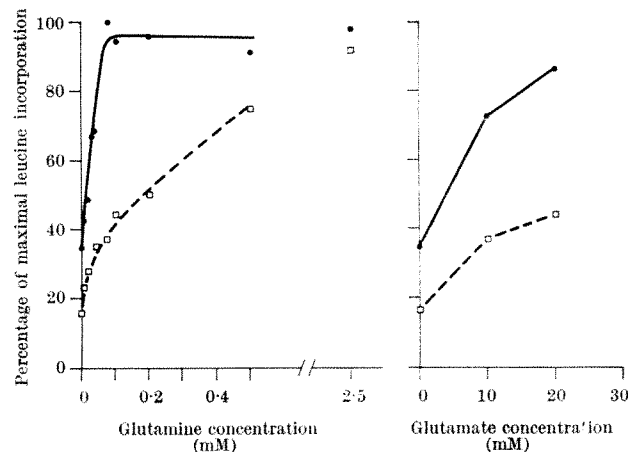


Fig. 1. Effect of L-glutamine and L-glutamate on incorporation of ¹⁴C-leucine into tumour cell protein. Washed Ehrlich cells (2.5×10^6 – 4.0×10^6 cells) were incubated at 37° C under O₂-CO₂ (95 : 5) in 2 ml. 'RMP1 1640' medium (Grand Island Biological Company) containing the indicated concentrations of asparagine, glutamine and glutamate. After 1 h, 0.5 μ Ci of uniformly labelled L-¹⁴C-leucine (311 mCi/mole) was added, and incubation continued for 1 h. Cells were harvested by centrifugation, washed, heated at 90° C for 15 min in 5 per cent trichloroacetic acid, collected on glass filter paper, and the radioactivity was determined with a liquid scintillation spectrometer. Maximal incorporation is the amount of radioactivity incorporated in a complete system containing 2.5 mM glutamine, 0.38 mM asparagine, and no glutamate. Glutamine was not added to incubation mixtures used for studying the effect of glutamate. —, With asparagine (0.38 mM); ---, without asparagine.

relatively high glutamine K_m (7×10^{-3} M) decreased the initial rate of growth of a number of tumours, including an Ehrlich ascites carcinoma, but caused no significant increase in the survival time of tumour-bearing animals. In this study, more intensive therapy with three extensively purified glutaminase preparations with considerably lower K_m values resulted in marked inhibition of an Ehrlich ascites carcinoma and significant increases in the survival time of tumour-bearing animals.

Ehrlich ascites carcinoma from Microbiological Associates (Bethesda) was carried by intraperitoneal injection of 0.2–0.5 ml. aliquots containing one million cells in 20–25 g Webster Swiss female mice from the Simonsen Company (Gilroy, California). The mice were treated with 270 IU L-glutaminase/kg/day for 16 consecutive days starting 24 h after tumour implantation. The enzyme was usually dissolved in 0.9 per cent NaCl 2–0.01 M sodium phosphate (pH 7.3) buffer before intraperitoneal injection of 0.1–0.4 ml. aliquots.

L-Glutaminase and L-asparaginase assays were performed in Tris-HCl buffer (pH 7.5) as previously described¹⁰ except that the colour formed by nesslerization of ammonia was measured 1–3 min after addition of Nessler's reagent whenever the incubation mixture contained glutamine. The methods used to determine blood amidase levels¹¹, asparagine synthetase¹², glutamyl-transferase⁵, protein¹³ (crystalline bovine albumin standard), pH-activity profiles¹⁰, and K_m values¹⁴ are described elsewhere. The method used for measuring the extent of incorporation of ¹⁴C-leucine into cell protein was essentially that of Sobin and Kidd¹⁵ except that ¹⁴C-leucine was in contact with the cells for only 1 h following a 1 h incubation of cells in non-radioactive medium.

Glutaminase preparations G1, G2, GA:1:2 and GA:2:0 were isolated from different bacteria by the enrichment technique¹⁶, G1 from a Gram-positive coccus and the other three from Gram-negative rods. The intracellular glutaminases were solubilized by ultrasonic disruption of cells and purified extensively by techniques commonly used for proteins^{10,17}. Glutaminase GA:2:0 was purified to an essentially homogeneous state, as judged by disc electrophoresis. The purified glutaminases were stable to lyophilization or storage in solution at 5° C for at

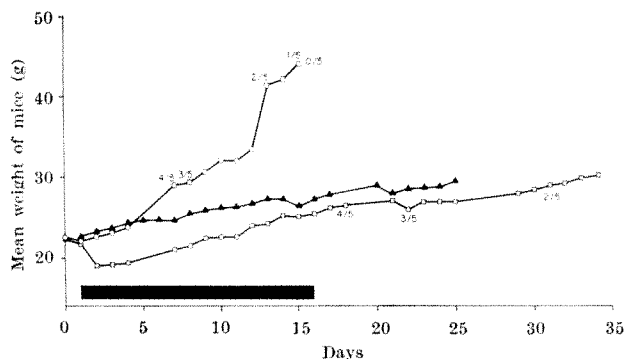


Fig. 2. Inhibition of growth of Ehrlich ascites carcinoma by highly purified glutaminase. Animals were injected intraperitoneally with 10^6 cells, and intraperitoneal treatment with glutaminase GA : 2:0 (■) was started 24 h later. Ratios listed on graph are (number of animals alive on designated days)/(total number at start of experiment). ▲, Normal mice; ○, tumour-bearing mice; □, enzyme-treated tumour-bearing mice.

least 1 week. A more detailed description of the purification steps and physicochemical characteristics of the glutaminases will be given elsewhere.

Several animal tumours were tested for glutamine and asparagine synthesizing activity. The high speed supernatant fraction of an Ehrlich ascites carcinoma homogenate had relatively little glutamyltransferase activity (5.5 nmoles/mg protein), by contrast with similar fractions of normal mouse brain, 585; liver, 432; kidney, 175; muscle, 34; and spleen, 17. The latter five tissue levels did not change significantly following two days of glutaminase treatment of normal mice. The Ehrlich tumour had a relatively high asparagine synthetase activity (7.9 nmoles/h/mg P). In the absence of glutamine, the Ehrlich ascites tumour exhibited marked reduction in the extent of incorporation of 14 C-leucine into cell protein (Fig. 1). The glutamine concentration required for maximal leucine incorporation was much higher when asparagine was removed from the medium. Furthermore, very high concentrations of glutamate (20 mM) substituted for the glutamine requirement only when L-asparagine was also present in the culture medium. Table 1 lists properties of the different bacterial glutaminases. All four enzymes have near-optimal activity in the physiological pH range, but vary in their K_m values (ranging from 4×10^{-6} to 6.5×10^{-4} M) and plasma clearance rates ($t_{1/2}$ 0.9–2.0 h).

Table 1. PROPERTIES OF VARIOUS BACTERIAL GLUTAMINASES

Preparation	G/A*	Spec. act. (IU/mg P)	K_m (M)	pH range of > 80 per cent optimal activity	$t_{1/2}$ (h)
GA : 1:2	1:2	135 (G)	2.0×10^{-4} (G)	5.5–9.3 (G) 5.9–10 (A)	0.9
GA : 2:0	2:0	150 (G)	4.0×10^{-6} (G) 8.0×10^{-6} (A)	5.8–9.2 (G) 8.5–10 (A)	2.0
G1	†	30	6.5×10^{-4}	7.2–8.8	1.4
G2	†	11	8.0×10^{-6}	7.2–9.3	n.d.

* Ratio of L-glutaminase/L-asparaginase activities.

† Asparaginase activity not detectable.

Repeated injections of the purified bacterial glutaminases were tolerated well by mice at a dose of 270 IU/kg/day for 16 consecutive days, and resulted in marked inhibition of abdominal distension and weight gain from tumour growth (Fig. 2). An increase in median survival time and long survival of tumour-bearing animals was seen in the glutaminase treated groups (Table 2). Heat-inactivated GA:2:0 and *E. coli* L-asparaginase did not significantly change the survival time of tumour-bearing animals.

The glutaminase-asparaginase preparations (GA:1:2 and 2:0) showed a greater antitumour effect than the enzyme with only glutaminase activity (G1). This difference may be the result of an enhanced requirement by Ehrlich cells for glutamine in the absence of asparagine (Fig. 1). It is also possible that the G1 preparation was

Table 2. EFFECT OF DIFFERENT GLUTAMINASES ON GROWTH OF EHRlich ASCITES CARCINOMA IN MICE

Preparation	Median survival time of mice in days (range)		Treated 30 day survivors	
	Control	Treated	Total	No tumour
G1	10 (7–16)	18 (14–30 +)§	1/5	1/5
GA : 1:2	10 (7–16)	> 30 (30 +)¶	5/5	3/5
GA : 2:0*	13 (7–16)	29 (18–60 +)¶	2/5	1/5
GA : 2:0†	12 (9–14)	11 (9–12)¶	0/5	0/5
EC-2‡	13 (7–16)	10 (9–20)¶	0/5	0/5

* Essentially homogeneous as judged by disk electrophoresis.

† Heat inactivated.

‡ *E. coli* asparaginase EC-2.

§ The significance of the increase in median survival time was $P < 0.05$; ¶ $P < 0.01$; ¶ $P > 0.25$ (not significant).

not as effective as the other two because of the higher K_m for glutamine. We plan to test these hypotheses by treating tumour-bearing mice with the pure glutaminases, G1 (higher K_m) and G2 (lower K_m) alone, and in combination with *E. coli* asparaginase. The GA:1:2 enzyme seemed to produce an antitumour effect at least as good as the GA:2:0 preparation, in spite of a higher K_m for glutamine and shorter plasma half-time. Further experiments are planned in order better to define the relationship between the physicochemical properties of the enzymes and antitumour activity.

When the tumour recurred in the animals treated with the GA preparations, it usually appeared after treatment was discontinued as a very slow growing subcutaneous tumour at a site of injection. An Ehrlich ascites tumour which recurred after treatment with glutaminase GA:2:0 (RAG-1), responded as well as the parent tumour to GA:2:0 treatment, in spite of an increase of glutamyltransferase activity from 5.5 to 16.9 nmoles/min/mg protein. Using glutaminase therapy with other neoplasms and other recurring Ehrlich tumours, we hope to establish the spectrum of sensitivity and define what relationship, if any, exists between glutamyltransferase activity, glutamine requirements for intracellular metabolism, and sensitivity to the amidase.

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Regulatory Factor for the Transcription of the Ribosomal Genes in Amphibian Oocytes

AMPHIBIAN oocytes provide very convenient material for the study of the mechanisms that control ribosomal RNA synthesis because their pattern of ribosomal RNA synthesis does not change greatly during oogenesis. During the lampbrush stage of oogenesis (stage 4) more than 97 per cent of the RNA synthesized per unit time in the oocytes is ribosomal. This happens because the genes for ribosomal RNA are specifically amplified³⁻⁵ to such an extent that the oocyte nucleus (germinal vesicle) has an rDNA content approximately 1,500 times more than the haploid amount⁴. On the other hand, in mature oocytes (stage 6) no ribosomal RNA is synthesized^{1,2}, although the extra copies of the ribosomal cistrons are still present. There must therefore be a mechanism in these cells which turns off ribosomal RNA synthesis. It should also be remembered that ribosomal RNA synthesis resumes only after the embryo has reached gastrulation^{6,7}. Furthermore, the cytoplasmic environment in the egg and in the pre-gastrula embryo is such that when nuclei from neurula endoderm cells, active in ribosomal RNA synthesis, are transplanted into enucleated eggs, they stop ribosomal RNA synthesis, although they synthesize DNA-like and soluble RNAs and are able to sustain normal development of the embryo. Ribosomal RNA synthesis in the nuclear transplanted embryos resumes only at gastrulation⁸. It has been suggested^{9,10} that this cytoplasmic control of the pattern of nuclear transcription could be mediated through a factor which specifically inhibits ribosomal RNA synthesis.

I have investigated the mechanisms responsible for the striking change in the pattern of ribosomal RNA synthesis during oogenesis, and have evidence for the presence in the mature metabolically inactive stage 6 oocytes of a protein factor with specific inhibitory effect on the transcription of the ribosomal genes.

Female *Xenopus laevis* were injected with 1 mCi of a ¹⁴C-amino-acid mixture lacking arginine and lysine 45 days after ovulation had been hormonally stimulated. After 3 days mature stage 6 oocytes were taken from the ovary and their germinal vesicles were isolated. (Details of these procedures will be published elsewhere.) The isolated germinal vesicles were homogenized at low

ionic strength (0.07 M) and the homogenate was centrifuged at a low speed and precipitated with ammonium sulphate. The fraction precipitated, which was between 35 and 45 per cent saturated and contained 53 per cent of the radioactivity incorporated into protein in the total homogenate, was redissolved in Alberts' buffer¹¹. When microinjected into stage 4 oocytes, this fraction had a strong inhibitory effect on the synthesis of all RNA species, possibly because of the presence of toxic contaminants.

The fraction was then applied to a DNA-cellulose column¹¹ prepared with *Xenopus* erythrocyte DNA enriched in ribosomal cistrons by polylysine precipitation of the A-T-rich sequences, according to Brown's technique¹².

Only a small part of the inhibitory activity in the ammonium sulphate precipitate bound to the column while the remaining activity was recovered in the eluate. Seventy-five per cent of the inhibitory activity bound to the column was eluted at 0.6 M NaCl. When injected into stage 4 oocytes this fraction inhibited RNA synthesis (measured as incorporation of ³H-uridine into trichloroacetic acid (TCA) precipitable material) by approximately 30 per cent. Hardly any specificity of inhibition of RNA species was detected when the RNA was extracted and analysed on sucrose gradients.

The 0.6 M fraction from the DNA-cellulose column was adsorbed to DEAE-'Sephadex' and stepwise eluted by increasing the ionic strength to 0.15, 0.3, 0.6 and 1.0 M KCl. When injected into stage 4 oocytes the 0.3 M fraction seemed preferentially to inhibit synthesis of ribosomal RNA. The 0.3 M fraction was then dialysed against 0.1 M KCl and rechromatographed on DEAE-'Sephadex' with a 0.1-0.3 M KCl gradient. Because it was practically impossible to assay each tube for effects on RNA synthesis, the tubes corresponding to optical density or radioactivity peaks were pooled and labelled as indicated in Fig. 1.

Each of the peaks in Fig. 1 was checked for its effect on RNA synthesis by microinjection into young stage 4 oocytes. After injection oocytes were allowed to incorporate ³H-uridine for 2 hours and then the RNA was extracted and analysed on sucrose gradients. To avoid complications due to possible effects on the processing mechanisms of the precursor molecule, only ³H-uridine incorporation into the 40S ribosomal precursor¹³ was taken as a measure of ribosomal RNA synthesis. It is, however, clear from Fig. 2 that amount of incorporation

into the 28S and 18S regions closely follows that into the 40S molecule. Of the other RNA species, only 4S RNA synthesis was followed, both because it is the only RNA species, apart from ribosomal RNA, that can be measured with reasonable accuracy in sucrose gradients and because it is known that 4S RNA synthesis is regulated independently of ribosomal RNA¹⁴. No attempt has been made to estimate incorporation into DNA-like RNAs.

Fig. 2 shows that when peak R material is injected into stage 4 oocytes, it strongly inhibits the incorporation of ³H-uridine into the 40S ribosomal precursor, while synthesis of 4S RNA is only slightly affected.

Table 1 summarizes the results obtained when the other peaks eluted from DEAE-'Sephadex' were used for injection. Peak R clearly contains a factor which specifically inhibits ribosomal RNA synthesis. I think that the 15 per cent inhibition of the 4S RNA synthesis obtained with peak R material, as well as the slight inhibitory effects of the other peaks on both 40S and 4S RNAs, are probably a consequence of unspecific damage to the cells.

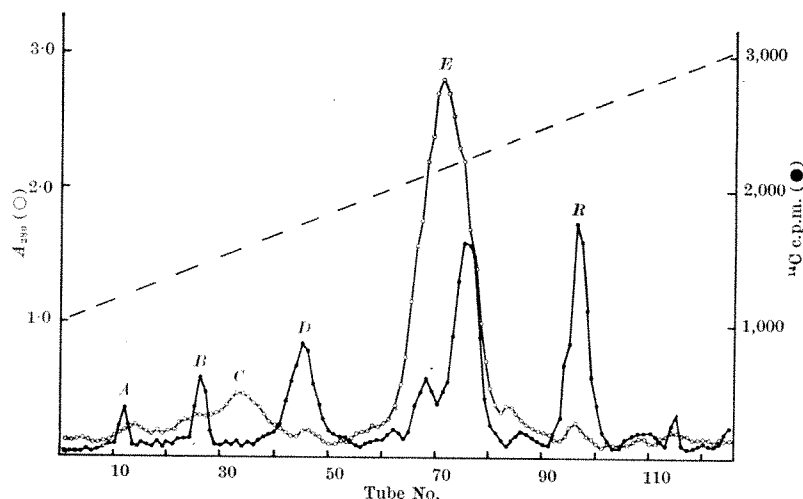


Fig. 1. DEAE-'Sephadex' chromatography of a fraction of the DNA-binding proteins from stage 6 oocytes. The 0.6 M fraction from the DNA-cellulose column was adsorbed to DEAE-'Sephadex' and stepwise eluted as described in the text. The resulting 0.3 M fraction was dialysed against 0.1 M KCl, 10 mM Tris (pH 7.4), 10 mM Mg²⁺, 6 mM mercaptoethanol, 0.1 mM EDTA, applied to a second DEAE-'Sephadex' column and eluted with a linear KCl gradient (0.1-0.3 M KCl). The loading and the elution were done at 4° C at a flow rate of 3 ml/h. The peaks were arbitrarily identified in the following way: A, tubes 10-14; B, tubes 24-29; C, tubes 31-38; D, tubes 41-45; E, tubes 62-83; R, tubes 91-101.

To substantiate further the specificity of the factor in peak *R*, its binding properties were investigated. Ribosomal RNA cistrons were isolated by the techniques of Brown¹² and Birnstiel¹⁵. In the same way I obtained DNA virtually free of ribosomal cistrons, as detected by hybridization with high specific activity *Xenopus* ribosomal RNA. The purified ribosomal cistron has a density of 1.724 and when hybridized with *Xenopus* ribosomal RNA showed a saturation of 20 per cent¹⁶.

Fig. 3 shows that the material in peak *R* had a striking binding specificity for the purified ribosomal cistrons. If the DNAs were previously denatured, binding was still observed but the specificity shown in Fig. 3 was partially lost.

It has been shown that the isolated ribosomal cistrons comprise some high-G-C "spacer" in addition to the sequences coding for the 28 and 18S RNAs^{17,18}, and so experiments are in progress to establish whether or not there is a preferential binding of the factor to any one of these components of the ribosomal RNA cistrons.

The inhibitory effect of peak *R* material disappears after trypsin digestion and heat denaturation and is resistant to ribonuclease. But although the phloroglucinol reaction for RNA¹⁹ gave negative results, a few ribonucleotides could be bound to the protein. By acrylamide gel electrophoresis the material is resolved into at least three bands, all moving to the anode but only one of them radioactive. In interpreting the binding results shown in Fig. 3 it should therefore be remembered that they apply only to the radioactive component of peak *R* material.

The results of control experiments rule out the possibility that the inhibitory effect is due to inhibition of the

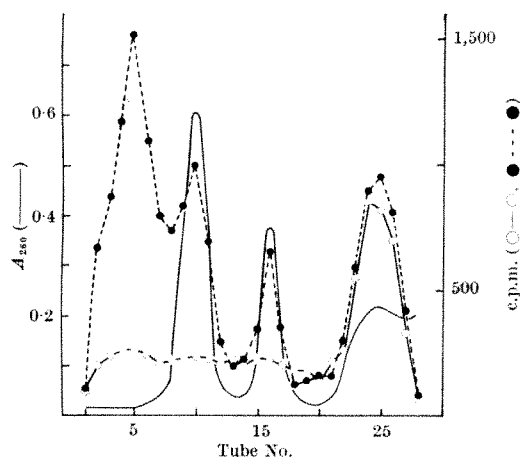


Fig. 2. Sedimentation profiles of radioactive RNA extracted from control (●---●) and treated (○—○) stage 4 oocytes. The control oocytes were injected with approximately 1/10 of their volume of Gurdon's saline buffer²⁴ (88 mM NaCl; 1.0 mM KCl; 0.82 mM MgSO₄; 2.4 mM NaHCO₃; 0.41 mM CaCl₂; 0.33 mM Ca(NO₃)₂; 2.0 mM Tris HCl, pH 7.6) containing ³H-uridine (0.06 μCi per oocyte). The treated oocytes were injected with the same amount of the same saline buffer containing, in addition to ³H-uridine, 1 mμg of peak *R* protein. Seventy-five oocytes were used for each gradient. After injection the oocytes were kept in Gurdon's saline buffer. At the end of the incorporation period (2 h) the oocytes were homogenized in a Dounce homogenizer. The RNA was extracted in 0.1 M acetate, pH 5.0 containing 0.5 per cent sodium dodecyl sulphate, precipitated with alcohol and layered over a 28 ml. 15–30 per cent sucrose gradient. The gradients were centrifuged for 18 h in a Spinco SW 25.1 rotor and collected. Absorbance was recorded by Gilford continuous recorder. All fractions were precipitated with TCA and counted in a liquid scintillation spectrometer (Nuclear Chicago). The radioactivity profiles of two gradients are superimposed; the first profile (●---●) was obtained with seventy-five oocytes which did not receive peak *R* material; the second profile (○—○) was obtained with seventy-five oocytes injected with 1 mμg each of peak *R* protein. Using actinomycin I established that at least 85 per cent of counts in the 4S region were a result of real synthesis and not terminal turnover.

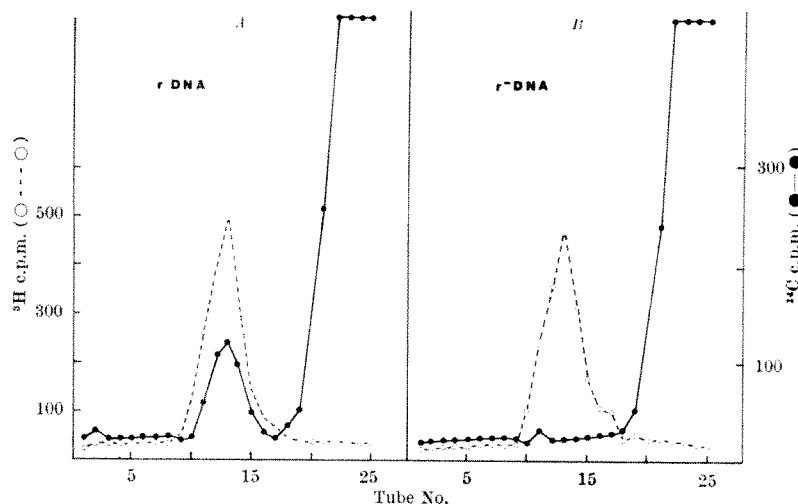


Fig. 3. Specific binding of peak *R* protein to purified ribosomal cistrons. Purified ribosomal cistrons (A) and DNA free of ribosomal cistrons (B) were prepared as described in the text. Both DNAs were tritiated. The ¹⁴C-labelled peak *R* protein was concentrated and mixed with rDNA or r-DNA. The incubation mixture contained, per ml.: 10 μg of DNA, 10 μg of protein, 50 μmoles of KCl and 1 μmole of EDTA. After 10 min of incubation at 37° C, 0.2 ml. of the mixture was layered on a 4.5 ml., 5–30 per cent sucrose gradient. After centrifugation for 4 h at 39,000 r.p.m. in a SW 39 Spinco rotor fractions were collected after puncturing the bottom of the tube. Each fraction was then precipitated with TCA and counted.

³H-uridine phosphorylation process or to a nuclease activity specific for ribosomal RNA.

When the extraction and fractionation procedures were repeated with germinal vesicles isolated from stage 4 oocytes active in RNA synthesis, the DEAE-'Sephadex' elution profile was different (my unpublished results) but no peak showed an inhibitory activity higher than 10–15 per cent.

The data discussed here seem to indicate that peak *R* contains a protein factor with regulatory properties specific for the transcription of the ribosomal cistrons. No conclusions, however, can be drawn about the mechanisms involved, but there are at least three possibilities: (1) the factor is a molecule with characteristics similar to a bacterial repressor^{20–22}; (2) the factor binds in some way to the RNA polymerase, thus rendering the enzyme inactive, or (3) the substance has the properties of an RNA polymerase subunit, synthesized during stage 6, incapable of initiating transcription of the ribosomal cistrons and recovered as an independent entity as a result of isolation and fractionation. There is evidence²³ that at least in stage 4 oocytes the rate of ribosomal RNA synthesis is controlled by the amount of RNA polymerase available. It should also be remembered that because of the unique features of oocytes, these cells could have special mechanisms which are not usually responsible for regulating ribosomal RNA synthesis in somatic cells.

Experiments are now in progress to test the effects of the factor on the *in vitro* transcription of the ribosomal RNA cistrons.

Table 1. EFFECTS OF DEAE-'SEPHADEX' PEAKS ON RNA SYNTHESIS

	40S RNA		4S RNA	
	c.p.m.	Per cent inhibition	c.p.m.	Per cent inhibition
Control	6,215	—	3,950	—
Peak A	5,519	11.2	3,389	14.2
Peak B	5,683	8.6	3,637	8.0
Peak C	5,625	9.5	3,411	13.7
Peak D	5,477	11.9	3,206	18.9
Peak E	5,289	14.9	3,271	17.2
Peak F	1,390	71.7	3,435	13.1

The tubes corresponding to the peaks indicated in Fig. 1 were pooled and their protein concentration was determined by Lowry's method. The fractions were then diluted to the same protein concentration and injected as described in the legend of Fig. 2. Oocytes were allowed to incorporate ³H-uridine for 2 h, and RNA was extracted and analysed on sucrose gradients. The radioactivity incorporated into the 40S and the 4S regions was precipitated with TCA and measured in a liquid scintillation spectrometer (Nuclear Chicago).

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they found that the F_1 hybrid animals derived from the mating of responder and non-responder parents gave an intermediate antibody response^{4,6}. This finding prompted us to compare the immune responsiveness of strain 2 "responder" guinea-pigs and F_1 (2×13) hybrid guinea-pigs to a DNP conjugate of a synthetic antigen (GL) controlled by the PLL gene.

F_1 hybrid guinea-pigs were produced by mating strain 2 and strain 13 guinea-pigs obtained from the National Institutes of Health Animal Production Section. Strain 2 and F_1 animals of identical sex and comparable age were immunized with 0.4 ml. of an emulsion composed of equal parts of DNP₃₃-GL in 0.15 M NaCl and complete Freund's adjuvant (Difco) which was distributed into four foot pads. Animals were immunized with either 0.01 mg or 0.1 mg of DNP₃₃-GL.

Thirteen days after immunization the guinea-pigs were skin tested with 0.01 mg of DNP₃₃-GL contained in 0.1 ml. of 0.15 M NaCl. Although there was some variation in the magnitude of the delayed response, there were no consistent differences between strain 2 and F_1 guinea-pigs (Table 1).

Table 1. DELAYED HYPERSENSITIVITY REACTIONS IN STRAIN 2 AND F_1 GUINEA-PIGS IMMUNIZED WITH DNP-GL

Strain	No. of animals	Immunizing dose of DNP-GL	Delayed hypersensitivity skin test with 0.01 mg DNP-GL at 14 days
2	4	0.01 mg	26.2* (20-35)
F_1	4	0.01 mg	19.8 (15-25)
2	3	0.1 mg	16.2 (10-20)
F_1	4	0.1 mg	26.3 (25-30)

* Average diameter in mm and range are given in parentheses.

Table 2. SERUM CONCENTRATION OF ANTI-DNP ANTIBODIES IN STRAIN 2 AND F_1 GUINEA-PIGS IMMUNIZED WITH DNP-GL, MEASURED BY PRECIPITIN ANALYSIS WITH DNP-BOVINE FIBRINOGEN

Strain	No. of animals	Immunizing dose of DNP-GL	Anti-DNP antibodies after 14 days (mg/ml.)	28 days (mg/ml.)
2	4	0.01 mg	0.46* (0.23-0.66)	0.71 (0.57-0.83)
F_1	4	0.01 mg	0.45 (0.37-0.52)	0.73 (0.51-1.05)
2	3	0.1 mg	0.88 (0.83-0.96)	1.8 (1.56-2.13)
F_1	4	0.1 mg	1.3 (0.95-1.47)	1.42 (0.83-2.3)

* Mean value and range are given in parentheses.

Guinea-pigs were bled 14 and 28 days after immunization. Anti-DNP antibodies were measured by quantitative precipitation with DNP₁₄₃-bovine fibrinogen as described by Eisen *et al.*⁷. Table 2 shows that the amount of antibody produced by F_1 and strain 2 animals is similar.

Table 3. ASSAY OF SERUM ANTI-DNP ANTIBODY LEVELS BY A MODIFICATION OF THE FARR TECHNIQUE IN STRAIN 2 AND F_1 GUINEA-PIGS IMMUNIZED WITH DNP-GL

Strain	No. of animals	Immunizing dose of DNP-GL (mg)	Per cent binding of ³ H-DNP-EACA by globulin fraction after 14 days*	28 days†
2	4	0.01	54.5‡ (34.2-65.5)	51.4 (34.2-65.5)
F_1	4	0.01	49.9 (47.7-54.6)	58.4 (45.6-63.7)
2	3	0.1	49.6 (39.3-63.3)	55.2 (37.8-65.7)
F_1	4	0.1	55.9 (48.8-63.9)	58.4 (38.5-72.5)

* Percentage binding of 0.1 ml. of 10^{-8} M ³H-DNP-EACA by globulin fraction of a 1/10 dilution of 0.1 ml. serum.

† Percentage binding of 0.1 ml. of 10^{-8} M ³H-DNP-EACA by globulin fraction of a 1/100 dilution of 0.1 ml. serum.

‡ Mean value and range are given in parenthesis.

Effect of Gene Dose on the Immune Response to a 2,4-Dinitrophenyl Glutamic Acid Lysine Copolymer

DOMINANT autosomal genes controlling specific immune responses to simple synthetic polypeptides of known composition have been described. In the guinea-pig, the immune response to poly-L-lysine (PLL), a copolymer of glutamine and lysine (GL), polyarginine, protamine and haptenic derivatives of these materials is controlled by the presence of the autosomal dominant, "PLL gene". As well as random bred Hartley guinea-pigs, in which the gene is variably present, there are inbred strains in which the gene is always present (strain 2) or always absent (strain 13). Strain 2 and Hartley guinea-pigs with the PLL gene are termed "responders" while strain 13 and the rest of the Hartley guinea-pigs are termed "non-responders". The (2×13) F_1 hybrid animal is heterozygous for the PLL gene and is always phenotypically a responder animal¹⁻³.

McDevitt and his associates have demonstrated that the immune response of inbred strains of mice to a related series of three multibranching polypeptide antigens is also controlled by an autosomal dominant gene (with multiple alleles), termed the "Ir-1 locus"^{4,5}. While investigating the immune response in these inbred mice,

Anti-DNP antibodies were also measured by a modification of the Farr technique in which the binding of ³H-DNP-EACA by the globulin fraction of the antisera was measured⁸. The data in Table 3 demonstrate no difference between F_1 and strain 2 guinea-pigs as detected by percentage binding of ³H-DNP-EACA by their sera. The fact that a ten-fold greater dilution of the 28 day sera gave approximately the same binding as the 14 day sera without a marked change in the amount of antibody present, is a consequence of the increase in binding affinity of the antibody with time after immunization. Because the F_1 and strain 2 sera behaved identically in this respect, it seems that F_1 and strain 2 animals produce antibodies of equivalent affinity for the DNP ligand.

Previous studies of these two immune systems have revealed many similarities³. The PLL gene in the guinea-pig and the Ir-1 gene in the mouse are both autosomal dominant traits controlling specific immune responses. In both systems it is possible to transfer the ability to respond from F₁ responder animals into lethally irradiated, non-responder parental recipients by the transfer of immunocompetent cells^{9,10}.

McDevitt and Chinitz have shown by breeding studies that the Ir-1 locus is closely associated with the major mouse histocompatibility locus (H-2) in the IX mouse linkage group⁵. We have investigated whether the PLL gene is linked to the major histocompatibility locus of the strain 2 responder animal. By back-crossing the heterozygous F₁ animal with the non-responder strain 13 animal, offspring were obtained which could be tested for both the presence of the PLL gene and the presence of the strain 2 histocompatibility antigen. The presence or absence of the PLL gene was always correlated with the presence or absence of the strain 2 major histocompatibility antigen¹¹. Thus both Ir-1 and PLL loci appear to be linked to the major histocompatibility locus in their respective species.

Our results demonstrate the first difference detected between the two systems. The F₁ hybrid mouse possessing the Ir-1 gene produces an antibody response intermediate between non-responder and responder parents. The F₁ hybrid guinea-pig possessing only a single PLL gene seems to have an immune response equivalent to its responder (strain 2) parent. Thus a gene dose effect is operative for the "Ir-1 gene" in mice which is not present for the PLL gene in guinea-pigs. Although the significance of this difference is not understood, analysis of gene dose effects in genetically controlled immune systems may provide insight into the mode of action of immune response genes.

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Vascular Hyper-reactivity with Sodium Loading and with Deoxycorticosterone Induced Hypertension in the Rat

HYPERTENSION may be induced in several species by excessive dietary salt, and is accentuated by the removal of a kidney and deoxycorticosterone administration¹⁻⁵. The blood pressure is raised because peripheral resistance is increased, but the exact mechanism leading to arteriolar narrowing is unknown. Vascular hyper-reactivity to pressor stimuli has been implicated^{6,7}, but when assessing this it is difficult to determine the relative importance

of true vascular smooth muscle hyper-reactivity, smooth muscle hypertrophy and minor changes in starting diameter of the resistance vessels^{8,9}. These problems can be largely circumvented by studying vascular reactivity in isolated perfused organs devoid of their normal nervous and humoral stimuli, and for this purpose we developed the vascular bed of the isolated perfused rat tail¹⁰. This preparation has a relatively simple structure; it includes terminal resistance vessels, and when perfused at constant flow rates with an artificial electrolyte medium containing dialysed bovine serum albumen, baseline resistance is stable for several hours. The tail responds with reproducible dose response curves to a variety of pressor agents.

Three groups of seven male Wistar rats were studied. Animals in one group were left intact and received a normal diet and tap water. Rats in the two remaining groups had one kidney removed and were given 1 per cent NaCl and 0.2 per cent KCl to drink. After two months, 12.5 mg deoxycorticosterone was given intramuscularly twice weekly to one of the nephrectomized groups. Systolic blood pressures were recorded in conscious warmed animals by tail plethysmography using a silicon strain gauge transducer. Pressure increased in the animals receiving deoxycorticosterone.

Perfusion studies were carried out 4 months after the original nephrectomy. By this time the nephrectomy/saline/deoxycorticosterone animals had been hypertensive for 4 weeks, and the day before tail perfusion studies, pressures were 177.9 (\pm s.d. 5.7) in this group compared with 120 (\pm s.d. 5.8) in the nephrectomy/saline animals ($P < 0.001$ for differences between means using Student's *t* test).

Animals were prepared for constant flow isolated tail perfusion¹⁰. Rats from the nephrectomy saline group were studied alternatively with those from the nephrectomy/saline/deoxycorticosterone group and before the untreated group. In each experiment two dose response curves to noradrenaline were obtained using bolus injections over a dose range 3–800 ng. The increase in resistance in response to noradrenaline was expressed as the change in pressure in mm Hg in the perfusion circuit proximal to the tail artery. Values from the two dose response curves to noradrenaline in each perfusion were averaged and these averages used subsequently.

The hypertensive deoxycorticosterone/saline/nephrectomy animals showed increased reactivity to noradrenaline at all doses (Fig. 1). Responses of the saline/nephrectomy group were intermediate to those of the hypertensive and intact animals. The differences were large, the average doses required to give a pressure increment of 50 mm Hg in tails from the three groups were 11, 66 and 170 ng in decreasing order of sensitivity. Wilcoxon's rank test was used to test the differences between groups as regards the doses required to give a 50 mm Hg pressure increment and was significant at $P < 0.01$ in each instance.

Responses to a single dose of 200 ng of synthetic angiotensin II amide ('hypertensin', Ciba) were recorded in the saline/nephrectomy and deoxycorticosterone/saline/nephrectomy groups only, and gave mean pressure increments of 11.7 (\pm s.d. 3.4) and 15.9 (\pm s.d. 9.1) respectively. This difference was not statistically significant ($P > 0.1$). Full dose response curves to angiotensin II were not obtained because of prolonged tachyphylaxis. Baseline resistance was not increased in preparations from the hypertensive rats and was slightly higher in the intact rats compared with the saline/nephrectomy group (Table 1). The intact group were also significantly heavier than the other animals at the time they were studied. As a further index of severity of blood pressure elevation heart weight was expressed as a percentage of body weight; values were greater in the deoxycorticosterone/saline/nephrectomy animals than in the other two groups (Table 1).

The increased reactivity of the hypertensive deoxy-

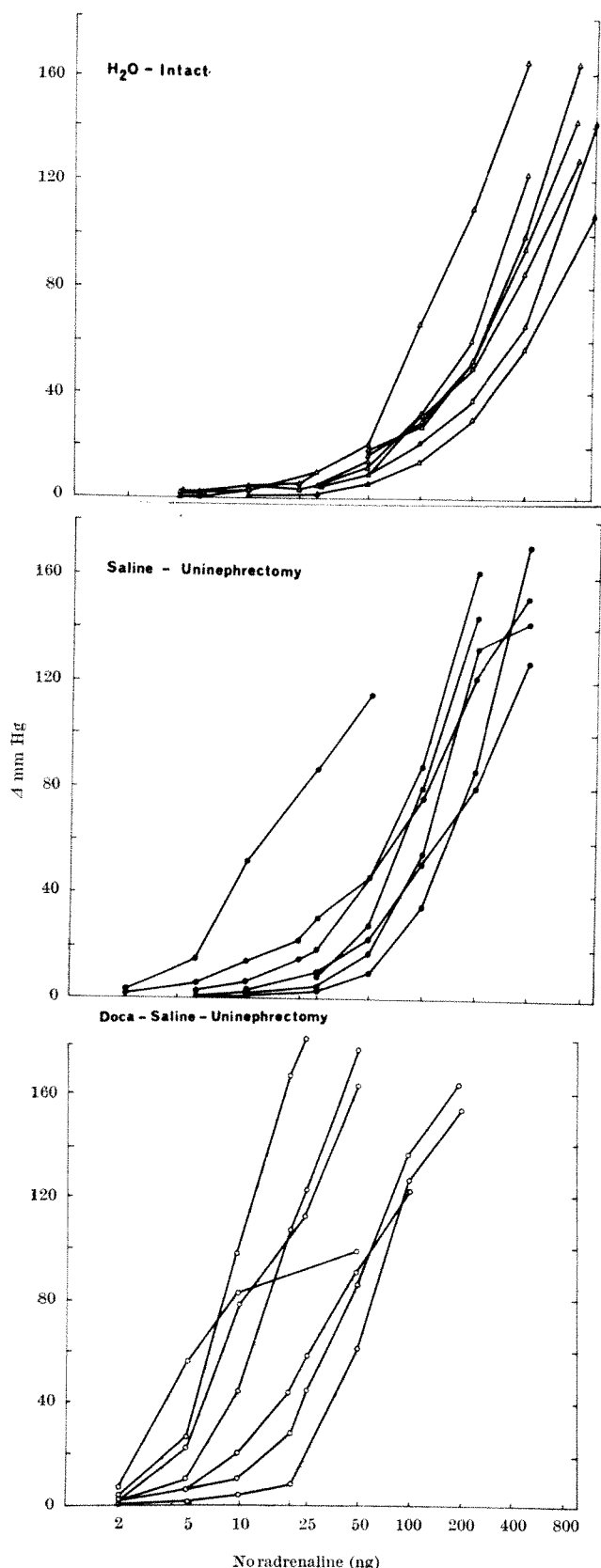


Fig. 1. Each curve represents the mean of two dose response curves to noradrenaline on each rat tail. The ordinate represents changes in pressure in mm Hg in the perfusion circuit in response to each dose of noradrenaline. Flow was constant at 0.5 ml/min with a bath temperature of 34° C. Increased vascular reactivity in the saline/nephrectomy rats (middle graph) and a further increase in the deoxycorticosterone/saline nephrectomy animals (lower graph) is shown.

corticosterone/saline/nephrectomy animals seemed to be specific for noradrenaline, and was not apparent when angiotensin II was injected. From the lack of significant differences in response to angiotensin we conclude that the large differences in response to noradrenaline did not result from vascular smooth muscle hypertrophy. This is further supported by the increased reactivity of the normotensive nephrectomy/saline animals compared with the untreated group. It therefore seemed likely that the increased reactivity either resulted from a change in the adrenoceptor, or from the local metabolism of noradrenaline. DeChamplain *et al.*¹¹ have shown that deoxycorticosterone and salt administration reduce noradrenaline metabolism by cardiac sympathetic nerves and they postulated that this occurred in arteries, and by accentuating the effect of autonomic nerve activity led to hypertension. This mechanism is unlikely to account for our experimental results, because although the magnitude of resistance change in response to noradrenaline increased with salt loading and deoxycorticosterone, the duration of response tended to decrease.

Table 1

	(Group 1) Untreated	(Group 2) Saline/ nephrec- tomy	(Group 3) Deoxycorti- costerone/ saline/ nephrectomy	P values comparing group means
Relative heart weight (per cent)	0.239 ±0.028 (6)	0.267 ±0.027 (7)	0.343 ±0.012 (7)	1 vs 3 <i>P</i> < 0.001 2 vs 3 <i>P</i> < 0.001
Total body weight (g)	549.4 ±41.3 (7)	426.6 ±72.98 (7)	370.0 ±34.75 (7)	1 vs 2 <i>P</i> < 0.01 1 vs 3 <i>P</i> < 0.001
Tail weight (g)	11.43 ±1.08 (7)	10.0 ±1.91 (7)	8.96 ±0.86 (7)	1 vs 3 <i>P</i> < 0.001
Baseline resistance (mm Hg)	22.57 ±3.60 (7)	17.43 ±3.95 (7)	18.71 ±2.75 (7)	1 vs 2 <i>P</i> < 0.05

Values are given as the mean ± s.d. Figures in parentheses are numbers of animals. Student's *t* test was used to compare groups. *P* values < 0.05 are shown in the right hand column.

In conclusion it has been shown that resistance vessels in the isolated perfused tail of animals treated by unilateral nephrectomy and salt loading show increased reactivity to noradrenaline and that this effect is accentuated in animals developing hypertension after administration of deoxycorticosterone. It is important to confirm that this change is specific for catecholamines and to establish whether there is a direct relationship between the altered vascular reactivity and the development of hypertension.

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Salsolinol, an Alkaloid Derivative of Dopamine formed *in vitro* during Alcohol Metabolism

THE Pictet-Spengler reaction is a special example of the Mannich reaction involving condensation of β -aryl-ethylamines with carbonyl compounds¹. In the case of phenylethylamines the resulting products would be 1,2,3,4-tetrahydroisoquinolines, which are particularly interesting because they could be formed from endogenous biogenic amines. The synthesis of simple tetrahydroisoquinolines in physiological conditions which occur in plants was described in 1934². The facility of this chemical reaction between dopamine and acetaldehyde in conditions which exist in both plants³ and animals^{4,5} has been confirmed. Because acetaldehyde is the primary metabolite of ethanol, it has been suggested that tetrahydroisoquinolines are formed in mammals after the ingestion of alcohol⁴⁻⁶. Indeed, this reaction forms the basis for the histochemical localization of catecholamines in nerve tissue for which formaldehyde vapour is used as the carbonyl agent. Reaction products of this type have been demonstrated in animals after administration of methanol⁷.

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is a condensation product of the neuroamine, dopamine, with the proximal metabolite of ethanol, acetaldehyde (Fig. 1). We have examined the formation of this tetrahydroisoquinoline alkaloid in rat liver and brain-stem homogenates. Because the intermediate metabolism of dopamine, ethanol, and acetaldehyde involves nicotinamide-adenine dinucleotide (NAD)-linked alcohol dehydrogenase and NAD-linked aldehyde dehydrogenase pathways, two tissues with widely differing capacities to oxidize alcohol or acetaldehyde were selected to determine the effect of rate of formation and metabolism of acetaldehyde on the formation of salsolinol. Incubation mixtures consisted of dopamine (0.2 μ Ci of ¹⁴C-dopamine plus 3.3 mM dopamine-HCl), acetaldehyde (0.5–4 mM) or ethanol (100 mM), and 1 ml. of tissue homogenate equivalent to 100 mg of rat brain-stem or 50 mg of rat liver. The total volume was 4.0 ml. In some experiments exogenous cofactor, (4 mM) NAD, was added. Incubations were carried out in 25 ml. Erlenmeyer flasks exposed to air in a shaking water bath for 30 min at 37° C. Ethanol

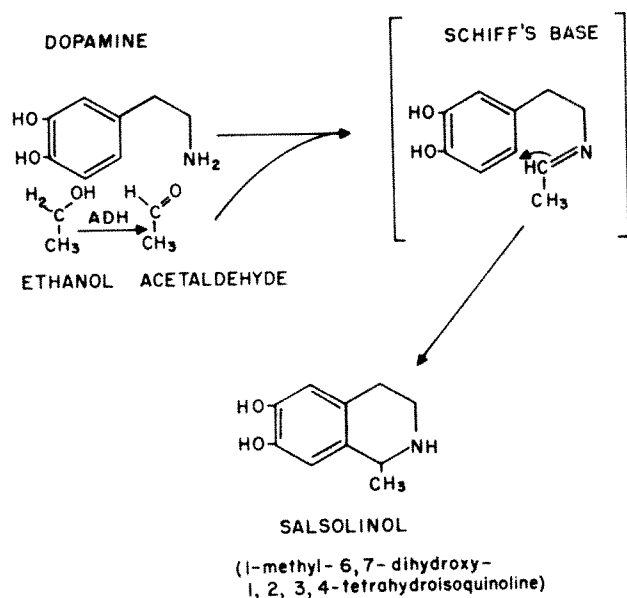


Fig. 1. Representation of the mechanism of formation of salsolinol by condensation of dopamine with acetaldehyde derived from enzymatic (alcohol dehydrogenase, ADH) conversion of ethanol.

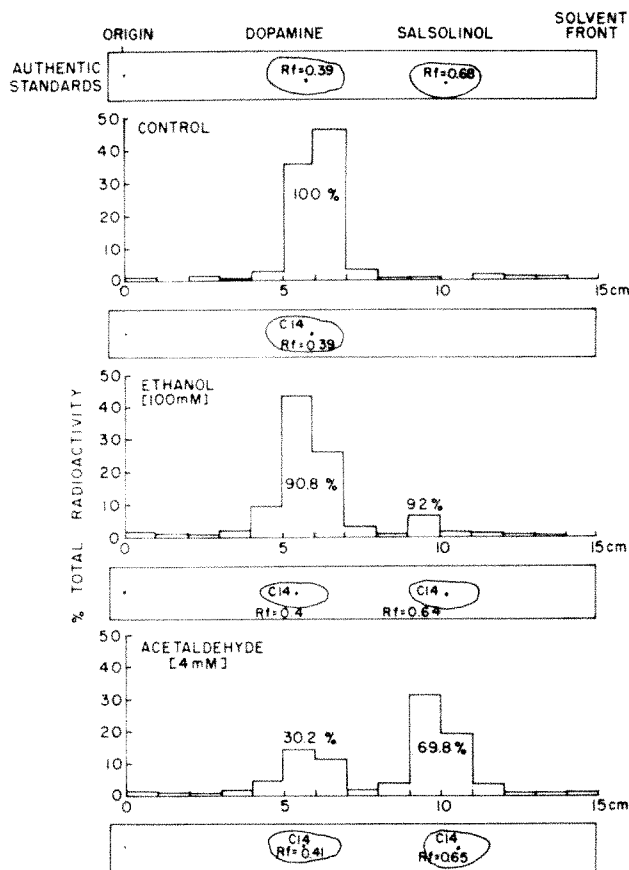


Fig. 2. Exact tracings of thin-layer chromatograms of authentic dopamine and salsolinol standards and ¹⁴C-dopamine and ¹⁴C-salsolinol isolated from rat liver homogenates. Histograms illustrate the effect of ethanol and acetaldehyde on the relative amounts of radioactive dopamine remaining and labelled salsolinol formed. Percentages and R_F values for experimental samples represent the means of fifteen determinations. R_F values for standard compounds are the means of twenty-five observations.

or acetaldehyde were added 1 min before the addition of dopamine.

Tissue homogenates were precipitated with 45 per cent perchloric acid and after removal of tissue protein and perchlorate salts, the extracts were adjusted to pH 8.4 and applied to columns of alumina (1 g of aluminium oxide, Woelm, neutral). Catechol compounds were eluted from alumina (7 ml. of 0.2 M HCl), adjusted to pH 2, and quantitatively transferred to four 100 mm columns of a strongly acidic cation exchange resin (Dowex AG-50W X 4, 100–200 mesh, H⁺ form). The catechol bases were adsorbed on this resin and completely separated from the deaminated catechols which passed through in the effluent. Two catechol bases (dopamine and salsolinol) were quantitatively eluted from the resin with 80 ml. of 0.5 M HCl. This procedure completely separates dopamine and salsolinol from the other catechol base metabolite of dopamine, tetrahydropapaveroline⁸. Dopamine and salsolinol followed almost identical elution patterns, but were readily separated and quantitated by thin-layer chromatography using cellulose Whatman 'CC 41' (Fig. 2). Chromatograms were developed in a mixture of chloroform, methanol and water (70:50:5) in the cold (4° C) for 15 cm under nitrogen, and spots were visualized by spraying with ferric chloride-potassium ferricyanide reagent. Authentic dopamine had an R_F of 0.39 and salsolinol standard an R_F of 0.68. Chromatography of the 'AG-50W' eluate in the presence of ethanol and acetaldehyde demonstrated two distinct and separate spots, identical with standard compounds (Fig. 2). The identity of ¹⁴C-salsolinol was further substantiated by gas-liquid chromatography⁹.

Table 1. EFFECT OF ETHANOL AND ACETALDEHYDE ON THE DISAPPEARANCE OF DOPAMINE AND THE FORMATION OF SALSOLINOL IN PHOSPHATE BUFFER, AND RAT BRAIN-STEM OR LIVER HOMOGENATES

Condition	Buffer		Brain (+NAD)		Liver (-NAD)		Liver (+NAD)	
	Dopamine	Salsolinol	Dopamine	Salsolinol	Dopamine	Salsolinol	Dopamine	Salsolinol
	(μ moles)	(μ moles)	(μ moles)	(μ moles)	(μ moles)	(μ moles)	(μ moles)	(μ moles)
Control	11.36 \pm 0.12	0	8.64 \pm 0.04	0	8.37 \pm 0.08	0	8.87 \pm 0.05	0
Ethanol 100 mM	9.99 \pm 0.51	1.65 \pm 0.51	9.83 \pm 0.06	0.20 \pm 0.06	8.65 \pm 0.22	0.61 \pm 0.22	9.97 \pm 0.24	0
Acetaldehyde 0.5 mM	9.24 \pm 0.16	2.51 \pm 0.16	7.69 \pm 0.04	0.89 \pm 0.04	7.41 \pm 0.13	0.87 \pm 0.13	8.09 \pm 0.13	0.51 \pm 0.13
" 1.0 mM	7.26 \pm 0.25	4.67 \pm 0.25	6.59 \pm 0.13	2.05 \pm 0.13	6.37 \pm 0.13	1.95 \pm 0.13	7.62 \pm 0.38	1.14 \pm 0.38
" 2.0 mM	3.94 \pm 0.19	7.89 \pm 0.19	5.17 \pm 0.02	3.62 \pm 0.02	4.76 \pm 0.17	3.74 \pm 0.17	6.49 \pm 0.22	2.44 \pm 0.22
" 4.0 mM	2.50 \pm 0.08	6.48 \pm 0.08	2.50 \pm 0.08	6.48 \pm 0.08	2.28 \pm 0.07	6.57 \pm 0.07	3.76 \pm 0.18	5.18 \pm 0.18

Values represent μ moles \pm standard deviation.

When ethanol was mixed with dopamine in phosphate buffer no salsolinol was generated (Table 1). Thus in the experimental conditions (30 min incubation at 37° C, pH 7.4) there was no spontaneous breakdown of ethanol to acetaldehyde. Addition of varying amounts of acetaldehyde, however, resulted in the *in vitro* synthesis of this tetrahydroisoquinoline alkaloid. The formation of salsolinol was directly proportional to the concentration of acetaldehyde when the concentration of dopamine was kept constant. The percentage radioactivity converted to salsolinol was 14.2–66.7 per cent in the presence of acetaldehyde (0.5–4 mM), not significantly different from the value obtained with brain-stem (+NAD) or liver homogenates (–NAD).

When 100 mM ethanol was added to incubation mixtures containing rat brain-stem homogenates and 16 μ moles of NAD, 2 per cent of the total radioactivity was converted to salsolinol (Table 1). The synthesis of salsolinol from ethanol in brain homogenates supports the finding of an alcohol dehydrogenase in the brain¹⁰, for this condensation product could only be formed if acetaldehyde were generated in the reaction mixture. When brain-stem homogenates were incubated with 0.5–4 mM acetaldehyde dopamine decreased (from 89.6 to 27.9 per cent) and salsolinol increased (from 10.4 to 72.1 per cent). Incubation of rat liver homogenates with 100 mM ethanol in the absence of NAD also resulted in the conversion of a small percentage (6.6) of the total radioactivity to salsolinol. Salsolinol could be formed only if ethanol were metabolized to acetaldehyde in these conditions. Even in the absence of added cofactor, sufficient endogenous NAD was presumably present to allow activity of liver alcohol dehydrogenase. Salsolinol production in rat liver homogenates (10.5–74.2 per cent of ¹⁴C) in the absence of NAD was proportional to the concentration of acetaldehyde, and closely paralleled that found when rat brain-stem homogenates were used in the presence of cofactor. This effect is probably due to the limited amount of NAD-linked aldehyde dehydrogenase in brain compared with liver¹¹. In the eluate from incubation mixtures of liver homogenate in the presence of NAD and 100 mM ethanol, all radioactivity was present in dopamine. The failure to form salsolinol in the presence of ethanol and cofactor may have been due to the rapid oxidation of generated acetaldehyde. In ethanol metabolism, NAD-linked alcohol dehydrogenase is the rate limiting enzyme¹², and so NAD-linked aldehyde dehydrogenase would quickly convert acetaldehyde to acetate and prevent it from condensing with dopamine. In the presence of NAD and acetaldehyde, salsolinol synthesis was reduced by 20–40 per cent. Thus the rapid oxidation of acetaldehyde by NAD-linked aldehyde dehydrogenase inhibited the formation of salsolinol by effectively reducing one of the requisite reactants, acetaldehyde.

Substantial salsolinol formation from dopamine occurred in both rat brain-stem and liver homogenates when acetaldehyde was added in concentrations approximating those which occur *in vivo*¹³. This isoquinoline alkaloid was also found in brain-stem and liver homogenates in the presence of ethanol (Table 1). It has been suggested that tetrahydroisoquinolines are formed from catecholamines as a result of ethanol ingestion and subsequent generation of acetaldehyde^{4–6}. Cohen and Collins⁶ reported that retrograde perfusion of adrenal glands with large

amounts of acetaldehyde resulted in the formation of tetrahydroisoquinolines derived from both noradrenaline and adrenaline. These condensation products had the same *R_F* values as the products of reaction between these catecholamines and acetaldehyde *in vitro*. The amounts of acetaldehyde perfused were far in excess of concentrations found after ingestion of alcohol. Such large quantities were probably necessary because this reaction with catechol-containing compounds which have substitutions on the side chain is quite slow. Robbins⁴ found the second order rate constants (l. mole⁻¹min⁻¹) for alkaloid formation (pH 7.4, at 37° C) between acetaldehyde and dopamine, L-dopa or noradrenaline to be 15.3, 6.1, and 1.9, respectively.

Thus this reaction is retarded appreciably when the ethylamine moiety contains substituent groups. The ease with which dopamine and acetaldehyde react (Table 1) indicates that the possible endogenous formation of an alkaloid after administration of alcohol would be most likely with this biogenic amine, especially in view of the short half-life of acetaldehyde *in vivo*¹⁴.

Tetrahydroisoquinoline alkaloids of the salsolinol type have been shown to have both central and peripheral pharmacological effects^{15–17}. These alkaloids may have either central nervous system depressant or stimulant and convulsant properties^{15,17}, vasopressor or depressor actions^{16,17}, various effects on smooth muscle^{16,17}, and lipid mobilizing activity¹⁸. It is intriguing to speculate that if tetrahydroisoquinoline alkaloids were formed *in vivo*, they might explain some of the pharmacological and behavioural effects produced by either acute or chronic ingestion of alcohol.

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Proteins of Rabbit Reticulocyte Ribosomal Subunits

By comparison with those of prokaryotic cells, especially *Escherichia coli*, little is known about the ribosomes of eukaryotes. The present analysis of ribosomal proteins from rabbit reticulocytes indicates that—in spite of the larger size of the particle and a higher protein content—the number of different proteins is not significantly greater than that in *E. coli* ribosomes. There may be multiple copies of some proteins and other proteins may be present in only a fraction of the ribosomes. Proteins from both ribosomal subunits are evidently considerably more basic than the corresponding proteins from *E. coli* subunits.

The amino-acid compositions of the total protein of the two subunits are strikingly similar (Table 1) as is indeed the case in *E. coli*¹. The ratio of basic to acidic amino-acids in reticulocytes is, however, 6 compared with 1.4 in the case of *E. coli*.

The multiplicity of ribosomal proteins from reticulocytes is clearly demonstrated by electrophoresis in polyacrylamide gels in conditions which have also been applied to *E. coli* ribosomal proteins (Fig. 1)². Some thirty zones for the large subunit and twenty for the small may be discerned. Similar numbers are reported for other eukaryotic ribosomes³ and *E. coli* ribosomes.

Gel electrophoresis in sodium dodecyl sulphate provides a means of estimating the molecular weights of all the separated proteins⁴⁻⁷. Fig. 2 shows that the resolution of ribosomal proteins is comparable with that in the conventional electrophoretic system: the large subunit gives eighteen major and ten minor zones, whereas the small subunit gives thirteen major and eight minor zones.

The relationship between electrophoretic mobility and molecular weight (Fig. 3) allows the derivation of molecular

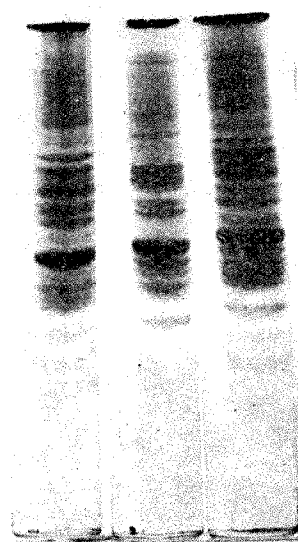


Fig. 1. Gel electrophoresis of ribosomal proteins from rabbit reticulocyte ribosomal subunits. To the ribosome solution (see Table 1) at 1–5 mg/ml. was added an equal volume of 4 M LiCl²⁴. The suspension was incubated at 0° C for 2 h and centrifuged at 600g to sediment the RNA. The supernatant was dialysed against 0.033 M sodium acetate and 6 M urea (freshly diazotized) (pH 5.6). Disc electrophoresis in 10 per cent polyacrylamide gels was performed in tubes of 6 mm internal diameter according to the method of Leboy and Flaks². The sample was composed of approximately 100 µg protein in 0.1 ml. solvent to which 0.01 ml. of a 50 per cent sucrose solution containing a trace of pyronine Y to serve as a tracking dye was added. Electrophoresis was carried out for 3 h at 5 mA/gel at room temperature in a Shandon electrophoresis tank. The gels were soaked overnight in 20 per cent sulphosalicylic acid and then stained for 2–4 h in a solution of 0.1 per cent Coomassie blue in water. The gels were electrophoretically de-stained and stored in 5 per cent acetic acid. These proteins have also been run in starch gel containing phenylacetic acid²⁴.

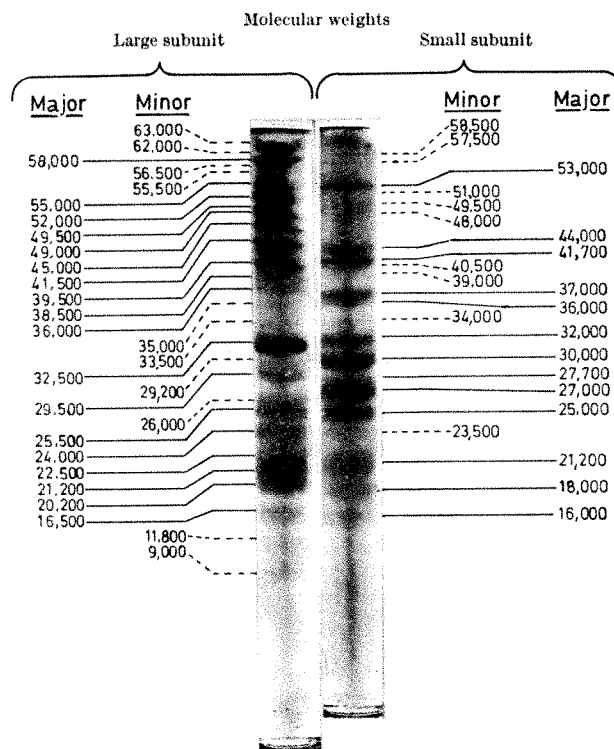


Fig. 2. Sodium dodecyl sulphate (SDS) electrophoresis of ribosomal proteins. The ribosomal subunits (Table 1) were made 1 per cent in SDS and dialysed overnight against a solution of 0.1 per cent SDS, 5 M urea (freshly diazotized), 0.1 M β -mercaptoethanol, and 0.01 M phosphate buffer (pH 7.6). To the sample containing 250 µg protein in 0.1 ml. was added 0.01 ml. of 0.002 per cent bromophenol blue in 50 per cent sucrose. Disc electrophoresis in 7.5 per cent polyacrylamide gels cast in tubes of 8 mm internal diameter and 20 cm length was performed as previously described⁴⁻⁷. Electrophoresis was at 5 mA/gel constant current at room temperature for 8 h with several changes of reservoir buffer using a Shandon disk electrophoresis tank. Gels were stained and de-stained as in Fig. 1.

weights for the ribosomal proteins in the range of 16,000–60,000. About half of the total number seem to have molecular weights greater than 40,000. All of the *E. coli* ribosomal proteins are smaller than 40,000 (refs. 8, 9).

Table 1. AMINO-ACID COMPOSITION OF RABBIT RETICULOCYTE RIBOSOMAL SUBUNITS

Amino-acid	Mole per cent of amino-acid	
	Large subunit	Small subunit
Aspartic acid	6.73	6.75
Threonine	3.73	4.42
Serine	5.00	4.54
Glutamic acid	8.40	9.88
Proline	4.20	3.67
Glycine	5.80	6.42
Alanine	6.92	5.88
Cysteine	7.20	8.14
Valine	5.45	5.76
Methionine	1.59	1.42
Isoleucine	4.20	4.32
Leucine	6.72	6.65
Tyrosine	2.14	2.12
Phenylalanine	2.73	2.73
Lysine	8.30	7.25
Histidine	2.02	1.89
Arginine	5.83	5.49
Ammonia	12.70	11.58

Rabbit reticulocyte ribosomes were dissociated with ethylenediaminetetraacetic acid as previously described²⁷. The fractions were concentrated by centrifugation at 105,000g for 16 h. The ribosomal pellets were redissolved in 0.025 M KCl–0.001 M MgCl₂–0.05 M Tris–10 per cent sucrose (pH 7.6) (medium A). RNA was extracted by the guanidinium chloride procedure²⁸. The ethanol supernatants containing the ribosomal proteins were dialysed extensively against water and the protein was lyophilized. Amino-acid compositions were determined with a Spinco amino-acid analyser after hydrolysis with 6 M HCl at 110° C for periods of 24, 48 and 72 h. Extrapolations for the labile residues were performed in the usual manner. The relative weights of the individual amino-acids except for cysteine and methionine agreed within 5 per cent in duplicate experiments. The relative weights of cysteine and methionine agreed within 20 per cent. Tryptophan was not determined but previous analyses indicate the amounts in the unfractionated protein is only about 1 per cent^{29,30}. This should not significantly alter the calculated relative molar composition. The mole per cent of acidic residues is calculated by adding aspartic acid and glutamic acid together and subtracting ammonia. The mole per cent of basic residues is calculated from the sum of lysine and arginine.

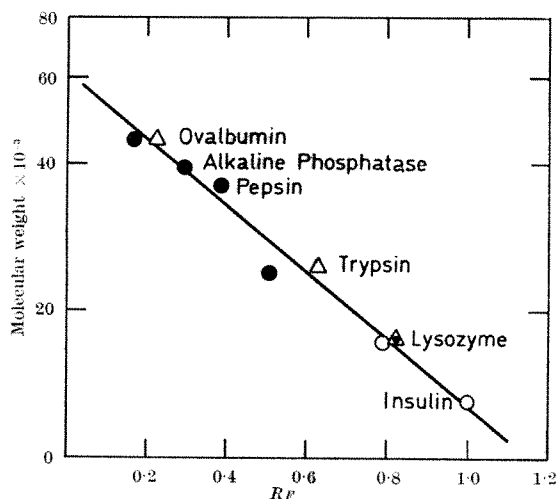


Fig. 3. Molecular weight calibration of SDS-polyacrylamide gels. Electrophoresis was carried out in 7.5 per cent polyacrylamide gels containing SDS as described in Fig. 2. 50 μ g samples of proteins of known molecular weight prepared as described for Fig. 2 were subjected to electrophoresis. The migration distances relative to the bromophenol blue dye are plotted against the logarithm of the molecular weight. The different symbols represent separate experiments.

The possibility that each electrophoretic zone represents a unique protein species present in every ribosome may be examined by three independent criteria:

(1) The sum of the molecular weights of the proteins leads to values of 0.66×10^6 – 1.0×10^6 for the protein of the large subunit and 0.41×10^6 – 0.8×10^6 for the protein of the small subunit (Table 1). Because the molecular weights of the RNA components of rat liver ribosomes are 1.6×10^6 and 0.6×10^6 (refs. 10, 11), respectively, the sum of RNA and total protein leads to values of 2.3×10^6 – 2.6×10^6 and 1.0×10^6 – 1.4×10^6 for the molecular weights and 29–39 and 40–58 per cent for the protein content of the subunits. The calculated maximum molecular weight of the large subunit falls well below the true molecular weight of the rat liver subunit, 3.1×10^6 , measured by equilibrium centrifugation¹¹, and the protein content is at most two-thirds of the measured value of 48 per cent. The calculated values of 1.0×10^6 – 1.4×10^6 for the molecular weight and 40–58 per cent for the protein content of the small subunit spans the measured values of 1.2×10^6 (ref. 10) and 52 per cent respectively.

(2) Because the area under the densitometer traces of the polyacrylamide gels stained with Coomassie blue should be proportional to the relative amount of protein¹², a gradation in the size of the peaks from high to low molecular weights would be expected. Fig. 4 does not bear out this expectation.

(3) The number of different proteins in the large and small subunits may be predicted by dividing the weight of protein in the subunit by the weight-average molecular weight of the unfractionated proteins of rat liver ribosomes, 26,000, measured by equilibrium centrifugation¹³. Thus the large subunit should contain fifty and the small subunit twenty different proteins.

A check on the average molecular weight of the proteins is obtained from these data in two different ways: First, assuming single copies of the proteins, values of 41,000–42,300, for the large subunit and 34,800–42,000 for the small subunit are calculated from the molecular weight distribution shown in Fig. 2. Second, making no assumptions and merely dividing the densitometer trace (Fig. 4) into halves of equal area values of 30,000 and 28,000, rather closer to the measured value 26,000 are obtained.

The observed discrepancies can only be explained if either some of electrophoretic zones are heterogeneous—unlike the case of *E. coli*—or if there are multiple copies of certain proteins in the ribosome. This situation is in striking contrast with *E. coli* where no more than one of each type of proteins is found, at least in the small subunit, and the expected number of different proteins is found in both subunits. It may be recalled that the RNA component of the large subunit of eukaryotic ribosomes is up to 60 per cent larger than that of *E. coli*¹⁴. *E. coli* ribosomal RNA seems to be derived at least in part by gene duplication^{15–18}. Additional binding sites for particular ribosomal proteins in the eukaryotic ribosomes could also have arisen in this manner.

As many as one-third of the ribosomal proteins of *E. coli* may be present in less than stoichiometric amounts in the ribosome⁹. Because the relative amounts of the proteins do not apparently vary with the growth conditions it is unlikely that the minor components are required to translate different sets of messenger RNAs. It has been proposed that they are involved instead in different stages of translation.

Reticulocyte ribosomes are concerned with the synthesis of essentially only two different proteins, the α and β -chains of haemoglobin, so that it is especially unlikely that the presence of numerous minor components reflects the requirements of specific messenger RNAs. Some of the minor components may reflect cytoplasmic contamination: this would explain the variation found between the proteins of the small subunit from different animal tissues¹⁹. Other proteins, as suggested previously, may be cyclically bound and released from the ribosome during protein synthesis.

The similarities in the mechanism of protein synthesis by *E. coli* and eukaryotic ribosomes, particularly as regards chain initiation, has been stressed in a number of recent publications^{20,21}. At least one important point

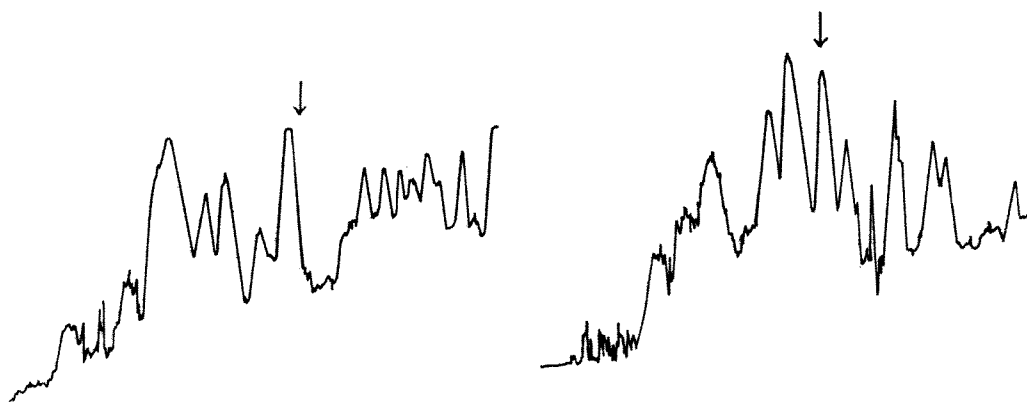


Fig. 4. Electrophoretic patterns of ribosomal proteins in polyacrylamide gels containing SDS. Densitometry of the polyacrylamide gels shown in Fig. 2 was performed using a Joyce-Loebel 'Chromoscan'. The scans were traced on heavy cardboard and cut at the position of the arrow into equal halves. This position indicates the average molecular weight by reference to Fig. 2 (Table 2).

Table 2. COMPARISON OF CALCULATED AND OBSERVED COMPOSITIONS OF RIBOSOMAL SUBUNITS

Subunit	Molecular weight of total protein			Percentage of protein by weight			Molecular weights of subunits			Weight average molecular weights of proteins			Number of proteins		
	Calculated*	Min- imum	Maxi- mum	Calculated†	Min- imum	Maxi- mum	Calculated‡	Min- imum	Maxi- mum	Calculated**	Min- imum	Maxi- mum	Calculated††	Min- imum	Maxi- mum
Large	655,900	1,038,900	1,500,000	29.0	39.4	48	2,255,000	2,638,900	3,100,000	41,000	42,300	30,000	50	18	28
Small	408,600	810,000	570,000	40.0	58.0	52	1,008,600	1,410,000	1,200,000	34,900	42,000	28,000	20	13	21

* Sum of the molecular weights listed in Fig. 2. The minimum value includes only the "major" zones. The maximum value includes the "minor" zones.

† Product of the observed fraction of protein by weight times the observed molecular weight of the subunit.

‡ Sum of the calculated molecular weights divided by the sum of the calculated molecular weights plus the observed molecular weight of the RNA component^{9,10}. Minimum value includes only the "major" zones of Fig. 2. Maximum value includes the "minor" zones.

§ RNA was estimated from the spectrum³¹ and protein by the Lowry procedure using bovine serum albumin as a standard (ref. 32 and unpublished results of H. J. G. and H. W. S. King). The validity of bovine serum albumin as a protein standard has been established for Jensen sarcoma ribosomes by comparison with the dry weight determination³².

|| Sum of the calculated molecular weight of protein and the observed molecular weight of RNA^{9,10}. The minimum value includes only the "major" zones, and the maximum value includes both the "major" and "minor" zones of Fig. 2.

¶ Molecular weights of rat liver ribosomal subunits determined by equilibrium centrifugation¹⁰.

** The sum of NM^2/M where N = relative number of protein copies, assumed to be one and M = molecular weight (Fig. 2). "Minimum" values include only the "major" zones. "Maximum" values include "minor" zones. The fact that the "maximum" values are greater than the "minimum" values is coincidental, arising from the fact that the "minor" proteins on the average are larger than the "major" ones.

†† Obtained by determining the point at which the densitometer trace in Fig. 3 could be cut into equal parts. The values agree with the figure of 26,000 previously obtained from the total ribosomal protein of rat liver¹⁰.

‡‡ The observed molecular weight of protein divided by the observed weight average molecular weight of the protein.

§§ The number of zones which appear in Fig. 2. The maximum value includes the "minor" zones whereas the minimum value does not.

does not seem to have been considered explicitly: the rate of protein synthesis in *E. coli in vivo* (15–30 amino-acids/s/ribosome^{23,24}) is more than ten times greater than in the two eukaryotic systems, chicken oviduct²⁵ and rabbit reticulocytes²⁶ which have been examined. That differences in function should be found is not surprising in view of the striking differences in the make up of the ribosome.

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Effects of L-Asparaginase on Lymphocyte-Target Cell Reactions *in vitro*

L-ASPARAGINASE was, until recently, thought to be a specific antileukaemic agent. It has recently been shown, however, to be an immunosuppressive agent, inhibiting antibody production¹, lymphocyte transformation^{2,3}, the graft-versus-host reaction⁴, and graft rejection⁵. We have investigated its effects in an *in vitro* system in which rat lymphocytes react to monolayers of mouse embryo cells, first undergoing transformation and then lysing the latter⁶.

Lymph-node cells from grey hooded rats, aged 1½–3 months, were suspended in Dulbecco's medium with 20 per cent horse serum and were added to Balb/C embryo fibroblast monolayers grown in 60 mm Falcon Petri dishes. Before adding the lymphoid cells, the monolayers were irradiated with 1,500 rads cobalt-60 γ-rays. The cultures were incubated for 5 days and the free-floating lymphoid cells were then passaged on to fresh monolayers. Lymphocyte transformation began at 3 days, newly formed blast cells proliferated and, during the 5th to 7th days, large pyroninophil cells (LPC) developed which completely lysed the target monolayer by the 7th day.

L-Asparaginase of *E. coli* origin (Farbenfabriken Bayer, A.G.) was dissolved in phosphate-buffered saline and added to cultures at a final concentration of 2.5 i.u./ml.; the solvent only was added to control cultures.

Asparaginase completely inhibited lymphocyte transformation and a practically pure population of small lymphocytes survived as long as asparaginase was present (up to 9 days in these experiments). In control cultures, transformation and lysis of the monolayer occurred. Fig. 1 shows the distribution of lymphoid cell sizes at the 8th day in cultures with and without asparaginase, the former showing a typical small lymphocyte population, the latter showing a mixture of small and large cells.

At the time of transfer of a lymphoid cell suspension on the 5th day, when numerous LPC were present, the suspension was divided into two parts, each being subjected to serial doubling dilutions. Asparaginase to a final concentration of 2.5 i.u./ml. was added to one series and all the suspensions were added to fresh monolayers

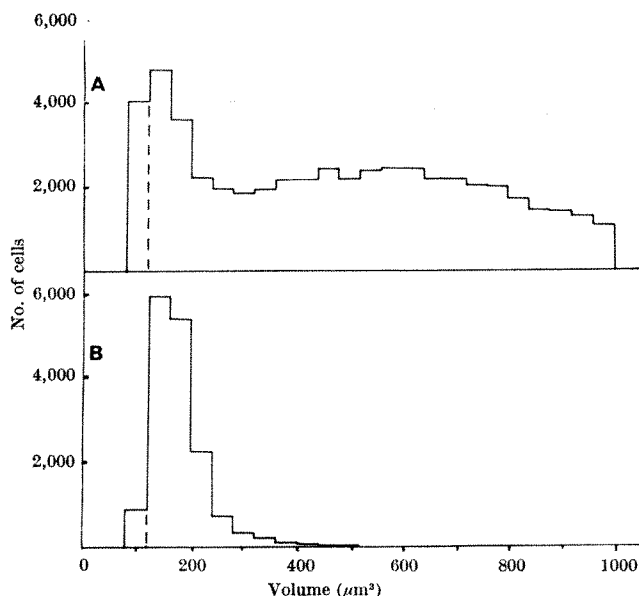


Fig. 1. Distribution of sizes of rat lymphoid cells cultured on mouse embryo monolayers. *A*, Rat cells in asparaginase-free medium, cultured 5 days, then transferred to a fresh monolayer and recultured 3 days; *B*, rat cells in medium containing 2.5 i.u./ml. asparaginase, cultured 5 days then transferred to a fresh monolayer in asparaginase-containing medium and recultured 3 days. Cell sizes were determined in a Coulter Model B counter. Note the large proportion of large cells in the control culture (*A*) and their absence from the culture made in the presence of asparaginase (*B*). The columns to the left of the dashed lines represent cell debris.

and were incubated for a further 2 days. Asparaginase inhibited lysis of the monolayer. Microscopy showed that all LPC in these cultures disappeared within 24 h, leaving a population of small and medium-sized lymphocytes.

In order to see whether the inhibitory effect of asparaginase was reversible, the lymphocytes from 5-day and 9-day cultures that had been grown in 2.5 i.u./ml. asparaginase from the onset were collected, centrifuged, resuspended in fresh asparaginase-free medium and were then added to fresh monolayers. Blast cells first appeared 3 days later and increased in numbers subsequently. They appeared to be morphologically normal but they did not develop into lytic LPC. Instead, transitional forms between blast cells and macrophages developed and, during the next 3-4 weeks, there was a progressive growth of macrophages which, mixed with medium-sized lymphocytes, eventually covered the monolayer confluent.

In summary, addition of 2.5 i.u./ml. of asparaginase to rat lymphocyte-mouse embryo cultures prevented transformation of the lymphocytes to large pyroninophilic cells, and a pure population of small lymphocytes survived as long as asparaginase was present (9 days). When asparaginase was then omitted, the cells that remained were unable to transform into lytic cells and many macrophages developed. When asparaginase was added to cultures in which transformation was actively occurring, the transformed lymphocytes were rapidly destroyed.

It therefore seems that, when rat lymphocytes are exposed to xenogenic cells in the presence of asparaginase, either their transformation into large pyroninophilic cells is prevented or cells that do begin to transform rapidly die and disintegrate so that they are not observed. The second interpretation is supported by experiments (unpublished) showing that, in cultures containing both asparaginase and phytohaemagglutinin, the lymphocytes disappear almost completely within 3 days, although their survival is excellent when phytohaemagglutinin is omitted. It is interesting to compare these results with those obtained when asparaginase is used to inhibit antibody production *in vivo*. In the latter case, the optimum time for adminis-

tration of the enzyme is during the 24 h or so before exposure to antigen; administration at later times, when the lymphoid cells are already undergoing proliferation, is ineffective¹. In *in vitro* conditions, however, cells engaged in transformation and proliferation seem to be highly susceptible to the action of the enzyme. The reason for this discrepancy is not clear. It is possible that the immunosuppressive action of asparaginase *in vivo* may be related to the ability, demonstrated *in vitro*, of limited exposure to the enzyme to inhibit lymphocyte transformation for a long period subsequently. It is to be noted that even when asparaginase was omitted from the medium after having been initially present in high concentration, the surviving lymphocytes were unable to undergo transformation subsequently, although morphologically they appeared healthy.

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Determination of *in vivo* Alterations in a Phenotype of Human Spermatozoa

A PHENOTYPE of rabbit spermatozoa for the dopa oxidase reaction has been reported¹. In the presence of L-dopa (dihydroxyphenylalanine), the cells develop melanin, proportional in amount or intensity to that present in the coat of the donor animal. These methods have been revised extensively to make possible a quantitative examination of the dopa reaction in human sperm.

Semen was taken from three donors selected on the basis of hair colour to increase the likelihood that they would vary in pigment genotype. Spermatozoa were separated from seminal plasma, washed in 0.9 per cent NaCl, and resuspended in Mann's Ringer solution². Glycerol was added to a sample of each suspension to provide a final concentration of 7.5 per cent v/v. Samples were cooled in the vapour of liquid nitrogen to 0° C in 60 s, and then to -196° C at an average rate of 200°/min. Experimental slides were incubated for 30 h in Sorensen's buffer (pH 7.4) to which was added 1:10,000 w/v of L-dopa. Two sets were reacted by the dopa oxidase method of Becker³, and one of these was subsequently stained for melanin by the hexamine-silver method of Gomori⁴. Control slides were processed through both reaction systems, but without L-dopa in the initial incubation.

After determining that Beer's law obtains in the dopa oxidase reaction, and that there is a definite absorption maximum for reacted cells, with controls having neither of these characteristics, the reaction in 100 cells per slide

was quantified by microspectrophotometry (my unpublished work); the plug method⁶ was used for the dopa oxidase reaction, at 405 m μ . Cells stained for melanin were studied by the two-wavelength method⁶, at 410 and 620 m μ . Calculations for the two-wavelength equations were made with the aid of the tables published by Mendelsohn⁷.

Table 1 gives the results of the work, with variance indicated by coefficients of variation. With respect to dopa oxidase activity in untreated cell suspensions, variance analysis gives $F_{2,297}=7.60$, $P<0.001$, and Tukey's test shows that each mean differs from every other. After freezing and thawing, there were significant differences among donors $F_{2,297}=7.22$, $P<0.001$, but in each case enzyme activity increased: $F_{1,594}=8.98$, $P<0.01$. A somewhat different pattern of alterations was evident in cells stained for melanin, after cryogenic treatment. Again, donors tended to differ with respect to their untreated cells, $F_{2,297}=2.85$, $P<0.05$, with those of T differing from those of H. But cells which were cooled to -196°C no longer displayed melanizing activities which differed among donors: $F_{2,297}=3.61$, $P>0.05$. The disappearance of mean differences among donors for melanin-stained cells may be a consequence of non-quantitative aggregation of silver particles on melanized cell surfaces in the hexamine-silver reaction. Coefficients of variation for both assays are most radically altered by treatment for the cells of the most deeply pigmented donor.

Table 1. DOPA OXIDASE ACTIVITY AND MELANIN REACTION IN HUMAN SPERMATOZOA; MEAN AND COEFFICIENTS OF VARIATION FOR 100 CELLS IN EACH CASE

Donor	Hair colour	Untreated	Dopa oxidase activity (M)		CV	Untreated	Melanin reaction (M)		CV
			CV	Frozen-Thawed			CV	Frozen-Thawed	
F	Black	1.9048	55.99	2.3315	86.77	286.42	32.39	306.85	40.34
H	Red	1.3979	54.09	1.5229	38.12	263.31	29.82	273.82	29.78
T	Blond	1.6965	89.10	2.0718	77.60	297.07	33.23	302.20	35.20

CV, coefficient of variation. M is given in arbitrary units^{6,8}.

The dopa oxidase phenotype is variable in the sperm cells of rabbit and man (ref. 1 and my unpublished work). It may be heritable, as are other variable sperm phenotypes⁸. The genetic basis of heritability might be haploid genetic activity in the cells during meiosis⁹, or alternatively, a function of the diploid genotype of the primary spermatocytes¹⁰. It is especially significant that cryogenic treatment can alter cell function in this system. Functional alterations have been found in association with the ABO phenotype of human sperm, which was first reported by Landsteiner and Levine¹¹ and by Yamakami¹²; these alterations are also a consequence of ultralow temperature treatment^{13,14}.

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New Ocular Hazard of Mode Locking in CW Lasers

In a series of experiments to investigate threshold retinal damage produced by CW helium-neon lasers (wavelength = 632.8 nm), we have found that lasers with identical beam divergence and output powers but different amplifier/cavity parameters produce different amounts of retinal damage. The source of this discrepancy has been traced to mode locking in one of the lasers used.

The pupils of eight adult Dutch rabbits were maximally dilated with 2 per cent homatropine before anaesthesia. One eye of each animal was exposed¹ to one or other of two laser systems. The whole of the laser beam entered the pupil, and the time of exposure was controlled by a shutter. Retinal damage, when present, was observed with an ophthalmoscope as small white spots. These lesions when examined under the electron microscope showed damage in the pigment epithelium and receptor cells.

The first laser was a B18/3 8 mW helium-neon (He-Ne) model loaned by Scientifica and Cook Electronics Ltd (London). This device is a d.c. uniphase laser with a 2 mm \times 580 mm cavity run single mode. The second laser was a standard G9 He-Ne laser supplied by International Research and Development Co. Ltd, Newcastle, and, for reasons other than those reported here, was run multimode to obtain the maximum output power. A laser such as the G9 with a long cavity and high gain can be made to mode lock by introducing in the cavity an absorption

cell of excited neon. When mode locked, instead of producing a continuous output, the laser generates a train of pulses at a very high frequency. Typically a He-Ne system operating in that way might produce pulses of width about 2 ns at a rate of about 75 million/s.

Loss mechanisms in a multi-mode laser cavity, other than an absorption cell, can also mode lock the system. As intra-cavity losses were introduced into the G9 laser to vary its output power, this output was examined for mode locking. The detector was a reverse biased PIN diode (Hewlett Packard 5082-4220, response time 1 ns) coupled to a Tektronix oscilloscope (Type 561A fitted with a sampling dual trace unit type 3S76 and a sampling sweep unit type 3T77A).

Fig. 1 shows that the G9 laser was mode locking and producing neither a continuous output nor random giant pulses (these latter would not form a trace with the sampling oscilloscope). The pulses are seen to have a pulse width of about 1.5 ns and occur every 11-12 ns. The apparent pulse height depends on the rise and decay times of the photodiode and oscilloscope, and on the noise which may trigger the sampling unit to produce a broad base-line.

The usual type of power detectors used to measure CW laser outputs are incapable of resolving such short pulses and will indicate only a mean power level. The peak power in a mode locked pulse will be greater than the mean power by the ratio of the pulse repetition period to the pulse half-width. Taking the values we have given, and assuming that the pulses are roughly triangular in shape, this ratio is approximately 8. In fact the ratio of peak to mean power could be higher than this because the pulse width may be less than 1.5 ns. Pulse widths as low as 10^{-11} s have been reported from mode locked laser systems but for a He-Ne laser it is unlikely that this width would be less than about 0.3 ns, making the maximum power ratio 35-40.

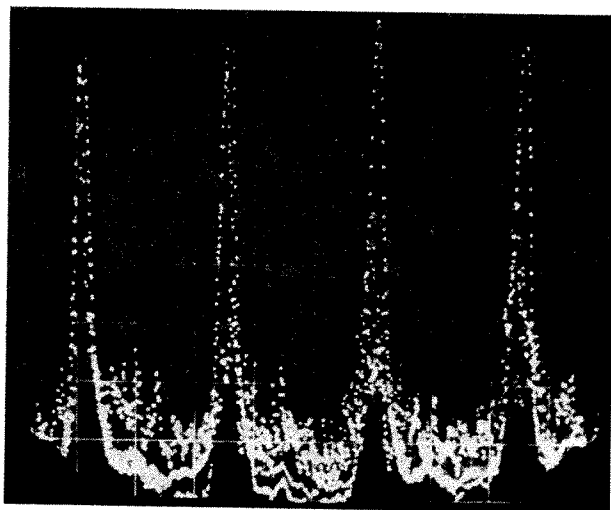


Fig. 1. Output of the unintentionally mode locked helium-neon laser described in the text. Oscilloscope record of photodiode output versus time. The mean power was 1 mW and the apparent peak power about 8 mW. The apparent half-width of the pulse was 1.5 ns and the repetition period 12 ns, 5 ns per large horizontal division.

Table 1 shows that when both lasers were operating at the same mean power it was possible to damage the retina only with the G9 laser though the energy dose was one twentieth of that from the B18/3 system. Herein lies the danger with a laser in which mode locking can occur. Pulsed laser studies have shown that the parameter which determines whether the retina is damaged is retinal peak power density²⁻⁴. With a mode locked laser the output of which is monitored with a conventional power meter the retinal power density may be an order of magnitude more than expected.

Table 1. COMPARISON OF UNINTENTIONALLY MODE LOCKED AND NON-MODE LOCKED HE-NE LASER RADIATION AND RETINAL DAMAGE IN DUTCH RABBIT EYES

	Mean power (mW)	Peak power (mW)	Exposure time (s)	Total energy (mJ)	Damage to retina
Mode locked, G9 laser	5	40-200	15	75	Minimal supra-threshold lesions
Non-mode locked, B18/3 laser	5	—	300	1,500	No lesions

All the parameters were measured at the cornea.

We wish to point out the potential hazard of laser systems which might unexpectedly be operating in the mode locked manner, especially because it is not clear whether the maximum permissible retinal exposure levels given in the various codes of laser practice⁵ are derived from systems that were or were not mode locking.

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Response to Criteria for the Designation of Persons working with Unsealed Radioactive Substances

HUGHES has outlined the derivation of criteria that he uses to decide if a worker using unsealed sources of radioactive material should be designated or not¹. Although I think that this general approach to the problem is appropriate, I would suggest that some of the numerical values of the parameters used in the derivation could be improved.

For the derivation of maximum activity to be handled at one time, in which intake of radioactive material resulting from an accident is considered the following points should be considered. (1) Hughes's premise that an individual will not be involved in such accidents more frequently than once per month led him to base his calculations on three-tenths of the monthly maximum permissible intake. Franke, Herrmann and Hunzinger² reported that only one radiation worker in five is likely to be involved in a radiation accident during a working life, so it would seem most restrictive to use the intake limit set by Hughes in this connexion. For designated persons the International Commission on Radiological Protection³ (ICRP), has stated that the maximum permissible quarterly dose should not exceed half the annual maximum permissible dose, and that if necessary this quarterly dose may be received as a single dose. By analogy, it would therefore seem appropriate to permit a non-designated worker a single intake of up to one half of three-tenths the annual maximum permissible intake, and I suggest that this is the figure that should be used in calculating maximum activity to be handled at one time.

(2) Based on the data of Franke, Herrmann and Hunzinger, Hughes selected a value of 10^{-5} for the parameter inhaled fraction of total activity handled (IFTAH). Donth and Maushart⁴ have published data which shows that in accidents involving gaseous or highly volatile radioactive substances, the IFTAH can be appreciably greater than 10^{-5} , and based on this data I would suggest an IFTAH value of 10^{-2} for gaseous and volatile sources, and 10^{-5} for all others.

When it comes to the derivation of maximum turnover per quarter, using small intakes from routine work as the basis of calculation, the figures derived by Hughes for radioiodines look unrealistically low compared with figures suggested in Publication No. 12 of ICRP⁵. This publication states that experience has shown that routine monitoring for internal contamination or for air contamination will rarely be needed, except when iodine isotopes at levels of a few hundred millicuries are handled. Other circumstances in which such monitoring might be required are also specified, but these are not relevant to conditions in universities and hospitals. The implication of this statement is that below such activity levels, intakes are most unlikely to exceed three-tenths of the maximum permissible intakes, and designation is therefore inappropriate. Hughes derived values of maximum turnover in experiments per quarter of 0.3 and 0.12 mCi for ¹²⁵I and ¹³¹I respectively, on the assumption that one hundredth of the activity used per quarter would be taken into the body. The ICRP guidance indicates that a more realistic intake fraction could be taken as 10^{-4} , and even this factor allows a reasonable margin of safety. I suggest therefore that a value of 10^{-4} be used for the fractional intake of the quarterly turnover, and that this value be used for all other radionuclides as well as for the radioiodines.

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Defence of Criteria for the Designation of Persons working with Unsealed Radioactive Substances

BUSH's comments (in the preceding communication) are a welcome development of the proposals in my earlier letter which are intended as a broad administrative guide capable of refinement if more precise levels of activity are to be set.

The maximum activity to be handled at one time would be increased by a factor of 6 if Bush's suggestion (1) is taken. But my original figures have not proved restrictive for most users in the University. Suggestion (2) makes a very important revision of the IPTAH in the case of gaseous and volatile substances and the figures for ⁸⁵Kr and ¹³³Xe in my letter should be adjusted accordingly.

Bush's suggestion for increasing the maximum turnover in experiments per quarter by a factor of 100 is inferred from the guidance of the ICRP¹ on the need to monitor individuals for internal contamination when handling gaseous or volatile materials and it would be very helpful if explicit recommendations could be made on this point. If the values are increased by a factor of 100, however, the need to provide individual monitoring for external radiation has to be considered because the activities of the sources will be appreciably higher than the levels set by the ICRP¹ for the introduction of such monitoring.

I am hoping that this correspondence will lead to the eventual inclusion in the appropriate codes of practice of further guidance on the levels at which designation is necessary.

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¹ *International Commission on Radiological Protection, Publication No. 12* (Pergamon, Oxford, 1969).

Marijuana and Memory

THE effects of marijuana are not consistent from subject to subject¹. Any discussion of its effect on human memory (such as refs. 2 and 3) must therefore consider whether valid generalizations can be drawn from the subjects who have been examined. One way of minimizing individual differences is to use subjects as their own controls, as is done in the present study which investigates the effects of marijuana on the recall of narrative material.

The subjects were eight men and women aged from 22 to 37. All but one were college students, and all had used marijuana several times before the experiment. Each was tested singly in two sessions separated by more than a month. In the first session, half of them were given marijuana and half acted as controls; in the second session, the roles were reversed. Those receiving the drug were given two marijuana cigarettes the tetra-hydro cannabinol content of which had not been ascertained. After smoking both, each subject was allowed to relax or read. Five minutes later the experimenter asked the subject whether he felt "high". All subjects answered affirmatively.

The experimenter, who remained in the same room throughout the test, then gave the subject a copy of Bartlett's *War of the Ghosts*⁴, and told him to read it through twice, at his own speed. When the subject had finished, the story was removed. Fifteen minutes later

the experimenter gave the subject a pen and paper and asked him to recall as much of the story as he could accurately remember, using the same words and phrases if possible. When the subject had finished, the session was over. The control subjects were put through the same test without taking marijuana.

The protocols were subjected to King's method of analysis⁵ which involves determining: (1) The total number of words in each subject's version of the story. (2) The number of "content words" with the exclusion of all articles, prepositions, conjunctions, and so on. Content words must have appeared in the original story, but can be misspelt or out of sequence. (3) The number of two word sequences in the recalls which had appeared in the original. (4) The number of correctly recalled four word sequences. (5) The number of "idea units", appearing in the recalls, as defined by a division of the original protocol into such units. Because this task required a certain amount of arbitrary judgement, the estimation was made by three arbiters who did not know the subjects or the conditions of the experiment. Their three scores were averaged to give the idea score for each recall. Table 1 shows the results of the analysis.

Table 1. COMPARISON OF RECALL MATERIAL UNDER MARIJUANA (M) AND IN CONTROL CONDITIONS (C)

Subject	Total words		Identical content words		Two word sequences		Four word sequences		No. of idea units	
	C	M	C	M	C	M	C	M	C	M
S ₁	227	214	66	66	45	30	9	5	52	42
S ₂	267	256	67	59	39	49	10	11	53	55
S ₃	234	155	61	27	41	17	9	1	50	28
S ₄	55	45	15	12	11	1	2	0	12	5
S ₅	170	159	42	30	31	17	6	4	34	26
S ₆	265	221	73	47	51	36	7	10	50	32
S ₇	215	197	63	51	39	34	9	6	40	40
S ₈	177	204	68	53	32	44	6	12	47	40
Total	1,610	1,451	455	345	289	228	58	49	338	268
Average	201.3	181.4	56.9	43.1	36.1	28.5	7.3	6.1	42.3	33.5
	$t=1.84$		$t=3.69$		$t=2.72$		$t=0.91$		$t=3.10$	
	n.s.		$P<0.005$		$P<0.025$		n.s.		$P<0.01$	

Seven of the eight subjects wrote less under marijuana than in the control condition. On the binomial test for one-sample cases⁶ this effect is significant at the 0.035 level. The difference between the total scores for two conditions, however, was not significant. When only the content words are considered, not only do the subjects perform worse under the influence of marijuana ($P=0.035$), but the number of recalled content words is also significantly less in this condition ($P<0.005$). The means were 56.9 and 43.1 for control and marijuana conditions respectively. The difference was evaluated by the t test for correlated means⁷.

With the two word sequences, only six subjects did worse under marijuana than in the control condition. This effect was not significant. When the total number of two word sequences in the two conditions is considered, however, the difference between the two means (36.1 and 28.5 for control and marijuana conditions respectively) is significant at above the 0.025 level.

The final index was the number of idea units in the recalls. Once again, subjects under marijuana tended to do worse but the trend was not significant. The means for the marijuana and the control conditions were 42.3 and 33.5 respectively ($P<0.01$), indicating that when there was an effect, it was significant.

The result of being "high" in this experiment was that subjects were not as capable of reproducing material which they had recently read. Not only were subjects worse at recalling exact words, they were also worse at recalling the ideas of the story they had just read. It is also noteworthy that the degree of impairment is significant. One can thus expect more than minor differences in behaviour as a result of smoking marijuana. The nature and direction of these differences await further study. But although individuals did do worse under marijuana, they were still able to read, remember and write when they needed to.

One difficulty with this type of research is in determining the locus of the effect on memory; the methodology made it impossible to know whether the same amount of information entered the memory faculties before the subjects were asked to recall the material. If this were so, then the effects of marijuana would not be on memory but on the input of information before it can enter the memory. On the other hand, the material may enter the memory stores equally well in control and in "high" conditions, and the difficulty may be with retrieval, as Weil and Zinberg suggest³. This problem could be answered by having subjects read the same material under marijuana and then asking some to recall it under marijuana while others recall in control conditions.

I thank Miss C. Edwards, Miss M. Coombs and Mr K. Stang for acting as arbiters. This study was conducted while I was a post-doctoral fellow at the University of California at Berkeley.

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Effect of Item Similarity on the Speed of Memory Search

Chase and Posner reported to the Midwestern Psychological Association in 1965 their investigation of the speed with which subjects determined whether a target letter (TS) was present or not in a comparison set (CS) when the letters were drawn from an acoustically similar population (AC), a visually similar population (VIS) or a neutral population (N), and the stimuli were presented visually. TS was presented either simultaneously with CS (visual comparison search), or before CS (visual recognition search) or after CS (memory search). From the evidence that verbal material is coded auditorily in memory^{1,2} they anticipated that acoustic similarity might affect speed in this task, changing the slope of the function relating reaction time and the number of letters in CS. In fact, only visual similarity had an effect, increased visual similarity increasing the reaction time in the visual comparison search and memory search. Chase and Posner concluded that a visually coded representation of the stimulus was used in these situations.

We have investigated whether acoustic similarity has an effect when stimuli are presented auditorily. The neutral and acoustically similar populations from Chase and Posner's experiment were used, with G replacing Z in the latter to allow for English pronunciation. The populations were A, D, H, I, M, Q, Y, Z (neutral) and B, C, D, E, P, T, V, G (acoustically similar). Only the memory search is possible when stimuli are presented auditorily. There were eight subjects, each serving in all six conditions. Conditions differed in the size of CS (1, 2 or 4) and the population of letters (N or AC). On each trial a CS drawn at random from the appropriate population was read out at a rate of two items per second, then a TS after a pause of 1 s. After a series of practice trials, four for each condition, the six conditions were given in random order with thirty-two trials in each condition. On half the trials TS was present in CS and in half it was not;

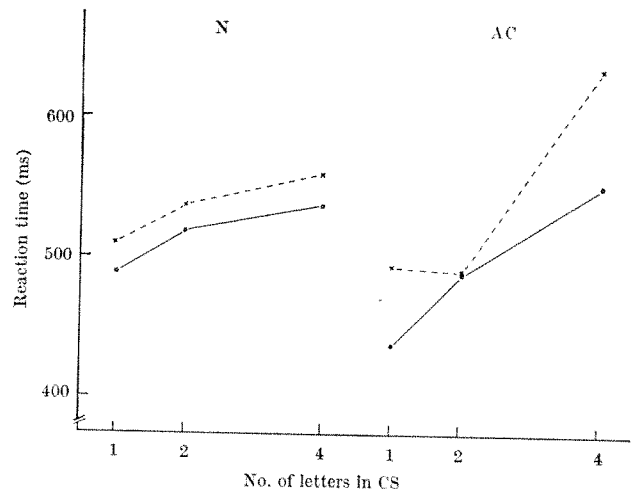


Fig. 1. Graph showing the mean time to decide whether a target letter was present or absent in a comparison set (CS) of letters, when letters were drawn from a neutral (N) or an acoustically similar (AC) population of letters. Results for positive responses (○—○) and negative responses (×---×) shown separately.

the two types of trial occurred in random order. Reaction time was measured by a timing device operated by a voice key; the timer started when the experimenter spoke the target letter and stopped when the subject responded "yes" or "no". If the subject made an error, the trial was rerun at the end of the condition.

The results were analysed by a four-way analysis of variance with each effect tested against its interaction with subjects. Fig. 1 shows that reaction time increased linearly with the size of CS (the linear trend was significant at the 0.001 level), and the increase was more rapid in the AC conditions (the difference in linear trend between N and AC conditions was significant at the 0.001 level). Negative responses were slower than positive ($P < 0.01$), which agrees with Nickerson's findings³ that mis-matches take longer than matches. This difference was greater in the AC conditions ($P < 0.025$), but Fig. 1 shows that this was not the case for all values of CS; in fact there was a significant triple interaction between stimulus similarity, size of CS and type of response ($P < 0.025$) so the interaction between stimulus similarity and type of response will not be discussed further. No other effect was statistically significant.

The result confirms findings such as those of Sternberg⁴ and Chase and Posner that reaction time in memory search is a linear function of the number of items in CS; this evidence suggests that TS is compared with each member of CS in turn. The result, compared with that of Chase and Posner, implies that the way stimuli are stored in memory in this task varies with the modality of the stimuli. The result does not, however, imply that each comparison of TS with a member of CS took longer when the items were acoustically similar than when they were from the neutral set. If this had been the case the function for the AC conditions would have been above that for the N conditions throughout, as in the result of Chase and Posner, whereas it was below that for the N conditions for CS1 and CS2. This implies that increased acoustic similarity speeded up this comparison process rather than slowing it down; therefore some other process must be sought to account for the change in slope associated with the change in acoustic similarity.

One possibility is that the subject decides not merely that some member of CS matches TS sufficiently closely to justify a positive response, but first decides which member of CS matches TS most closely, then decides whether a positive response is justified. To do this, every member of CS must be examined, even when a close match of TS with a member of CS is found early in the series of comparisons. That this is done is implied by the similar

slopes for positive and negative responses, as Sternberg⁴ pointed out. If the time taken on this decision, as to which member of CS matches TS most closely, is a linear function of the size of CS, and if the decision is more difficult and takes longer when the items are drawn from a similar population than when they are drawn from a neutral population, then the obtained result may be deduced.

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Eye to Eye Transfer of an Early Response Modification in Chicks

ZEIER¹ reported that trained loss of a cliff-avoidance response did not transfer from eye to eye in the chick whereas imprinting and pattern discriminations do transfer in birds. He suggested "It is possible therefore that tasks involving responses for which there is a strong inherent disposition are more easily transferred from eye to eye than are reactions that run counter to an innate response tendency". I have observed, however, transfer of a learned response that runs counter to the "innate" tendency of chicks to peck small targets.

The basic procedure has been described^{2,3}. When neonate white Leghorn cockerels are presented with a small target, nearly all peck it within 10 s. Repetition of this response can be suppressed by a single presentation of the target coated with an aversive liquid such as *n*-propanol⁴ or methyl anthranilate^{2,3,5}. I have used this response modification to study transfer by training chicks with one eye occluded followed by testing after 16–128 min with the contralateral eye occluded.

The original occluder was a Band-Aid "sheer spot" (Johnson and Johnson), a 22 mm circular adhesive plastic with a 10 mm central gauze pad; the pad was centred over the eye. In a second experiment two "sheet spots" were overlapped to a long diameter of 26 mm. Each occluder was inspected before each target presentation and pressed down or replaced as necessary. Each monocular occluder, whether shifted or not, was replaced with a fresh occluder before the test trial to equalize handling conditions. Chicks tested binocularly wore a monocular "occluder" left open rostrally to permit vision; it was also replaced. The ability of "sheer spots" to block vision was confirmed by presenting the target to chicks ($n=40$) with both eyes occluded. No pecking occurred in 97.0 per cent of 200 presentations; the comparable rate for chicks with monocular or binocular vision was 8.3 per cent.

The training target, a 3 × 5 mm microminiature lamp, was dipped into methyl anthranilate just before presentation to each experimental chick or into water before presentation to each control chick. The lamp was dry during all test presentations. The novel test target was a dry 3 mm stainless steel bead⁵. The chicks were individually blind-coded between training and testing. The interval between training and testing was 16, 32, 64, or 128 min in the first experiment; the results were independent of the interval and are pooled in Table 1 (groups 1–4). A single training-testing interval of 120 min was used in the second experiment (Table 1, groups 5–10). Chicks

Table 1. DISCRIMINATED TEST RESPONSE OF CHICKS TO THE TRAINING TARGET (LAMP) AND A NOVEL TARGET (BEAD)

Group	Condition	Train-test interval (min)	Test response					
			Lamp			Bead		
			A	P	Per cent A	A	P	Per cent A
1 Exp.	L-R	16-128	31	9	77.5	7	33	17.5
2 Exp.	R-L	16-128	29	11	72.5	6	34	15.0
1,2 Exp.	L-R, R-L	16-128	60	20	75.0	13	67	16.3
3 Control	L-R	16-128	8	32	20.0	0	40	0.0
4 Control	R-L	16-128	6	34	15.0	2	38	5.0
3,4 Control	L-R, R-L	16-128	14	66	17.5	2	78	2.5
5 Exp.	L-R, R-L	120	17	3	85.0	3	17	15.0
6 Exp.	L-L, R-R	120	13	7	65.0	0	20	0.0
7 Exp.	Binocular	120	11	9	55.0	1	18	11.1
8 Control	L-R, R-L	120	2	18	10.0	0	20	0.0
9 Control	L-R, R-R	120	3	17	15.0	0	20	0.0
10 Control	Binocular	120	3	17	15.0	2	18	10.0

Experimental groups (Exp.) were trained to avoid the lamp by coating it with methyl anthranilate; control groups were "trained" on the lamp coated with distilled water; L, left; R, right; L-R condition signifies training with left eye open, testing with right eye open; R-L condition signifies the reverse; L-L and R-R signify training and testing with the same eye; A, avoided target; P, pecked target.

trained monocularly transferred the peck avoidance response to the contralateral eye (groups 1, 2 and 5). In the first experiment (groups 1 and 2) 75 per cent of chicks trained monocularly avoided the lamp target when tested with the untrained eye; in a separate experiment² with normal binocular training and testing, the mean avoidance was 74.5 per cent and the range was 62.5 to 85.0 per cent in twelve groups ($n \approx 40$ per group). In the second experiment, chicks tested with the untrained eye (group 5) had a higher avoidance than chicks tested with the trained eye (group 6) or binocularly (group 7) but the differences were not statistically significant ($\chi^2=1.20$, $P>0.2$; $\chi^2=2.98$, $P>0.08$, respectively). There was no evidence of significant lateralization; the pooled avoidance of all L-R chicks (trained with the left eye, tested with the right eye) did not differ significantly from the pooled avoidance of all R-L chicks (41/50 versus 36/50; $\chi^2=0.90$; $P>0.3$). The test with the novel bead target ruled out a general performance deficit as a factor in the avoidance response; the results demonstrate that peck performance *per se* was normal, because 82.5 to 100 per cent of each group pecked the bead.

The successful interocular transfer of trained loss of "innate" pecking behaviour runs counter to the generalization suggested by Zeier¹. Possibly the explanation is to be sought in one of the other reported determinants of interocular transfer in birds, for example position of the relevant stimulus in the visual field⁶.

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Faeces of the Medicinal Leech, *Hirudo medicinalis*, are Haem

The pigmented tissue around the gut of the blood feeding leech, *Hirudo medicinalis*, was regarded by Moquin-Tandon in 1826 (ref. 1) as a liver. Spiess² challenged this concept on the grounds that the pigmented tissue was not

derived from the gut, but he agreed that it was a functional liver even if it was not a morphological or an embryological one.

Spiess examined the faeces chemically and concluded³ that bile pigments were excreted in the faeces, further confirming his belief that this tissue was indeed analogous to the vertebrate liver. He presented⁴ a new theory of the evolution of the leech liver based on his identification of bile pigments. Fukui, apparently unaware of Spiess's work, identified⁵ the faeces as haematin and could not detect bile pigments or haematoporphyrin in the faeces or even in the whole leech. He concluded that the ingested haemoglobin is split into globin, which is digested, and haematin, which is excreted in the faeces.

I have attempted to resolve this conflict by starving *H. medicinalis* and then by feeding them on rat blood. The faeces were collected and assayed. The aqueous solution of fresh faeces was green and had an absorption spectrum with a maximum at 397 nm and plateaux of 565 (broad) and 605 nm. When made to 1.0 N HCl, the solution turned reddish brown and absorbed at 394 and 655 nm with plateaux at 520 and 550 nm. When made to 1.0 N NaOH, the solution turned green again and absorbed at 390 and 590 (broad) nm. The peaks at 397, 394 and 390 nm were very narrow and much more intense than the others, qualifying as Soret bands, a characteristic of all tetrapyrrole rings, that is, porphyrins⁶. Illumination with ultraviolet through a Wood's filter (365 nm) did not elicit a fluorescence in acid, neutral, or basic solutions of faeces. Thus the porphyrin is not "free" but has its pyrrole nitrogens completely bound. Tests for iron in the faeces were negative unless the samples were ashed or treated with reagents, such as NaOCl or H₂SO₄, which liberate the iron. Iron was detected using KCNS, K ferrocyanide, and thioglycolic acid⁷. A porphyrin containing bound iron is a haem; therefore, the faeces contain a haem.

The presence of a haem was confirmed by the emission of a bright red orange fluorescence under ultraviolet illumination after treatment with concentrated H₂SO₄ which liberates the iron and frees the porphyrin⁸. The same fluorescence was also obtained using acetic acid with sodium hydrosulphite⁹. Further confirmation was obtained by converting the faeces to pyridine haemochromagen⁶. No bile pigments were detected in the faeces using the Gmelin reaction⁹ and the sensitive Schlesinger reaction¹⁰. The faeces seem to consist almost entirely of haem. Tests for protein, reducing and non-reducing carbohydrates, and lipid were all negative to at least the 1 per cent level.

Spiess's results can be explained by appraising his methods³. *H. medicinalis* only defaecates occasionally, from once a week to once a month. In order to obtain samples of a sufficient size, he collected the faeces over a period and concentrated the sample by prolonged boiling. This heat treatment cleaved the tetrapyrrole ring, liberated the iron and did, in effect, leave him with bile pigments.

In conclusion, my investigations show that the faeces of *H. medicinalis* are a haem. Fukui was right and Spiess was incorrect. The leech system described here is presented as a model for blood feeding animals. More important, perhaps, is that *H. medicinalis* is defaecating almost pure haem.

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Capric Acid as a Larvicide and an Oviposition Stimulant for Mosquitoes

CONCERN about the excessive use of insecticides has focused attention on less toxic materials and revived interest in certain fatty acids¹⁻⁴. Insecticidal properties of some of these acids (especially of their potassium soaps) have long been recognized and this has led to their widespread use in sprays^{5,6}.

During a field assessment of capric (decanoic) acid as a mosquito larvicide, a potentially more valuable entomological use for this acid was found: as well as being a larvicide, its addition to artificial pools⁷ made them strikingly attractive oviposition sites for the mosquito, *Culex restuans* Theobald.

The larvicidal activity was evident after capric acid was added in amounts equivalent to 150 and 300 p.p.m. each to five pools randomly selected along with six untreated pools or controls in a four by four array. Larvae died in 3 days after treatment at a mean maximum temperature of 26° C and in 14 days at 10° C. During this same period the pools were unacceptable to mosquitoes as oviposition sites. This "repellent" period was followed by several successive phases with distinct characteristics: (1) pools gradually lost their repellency and became acceptable oviposition sites; (2) pools became exceptionally attractive for oviposition; (3) treated pools gradually became no more acceptable than untreated pools. By this time the treated pools, which during phase (2) had turned opaque brown, had lost their colour and became as clear as untreated controls. Maximum acceptance for oviposition occurred 10-14 days after application of 150 p.p.m. and 14-21 days after application of 300 p.p.m. of capric acid. After the "repellent" period the treated pools were no longer lethal to mosquito larvae or other arthropods.

During 2 months after treatment 2,866 egg rafts of *C. restuans* were deposited in ten treated pools and only five rafts in six untreated pools.

Pools treated with capric acid but containing no detectable, or very few bacteria of the family Pseudomonadaceae (identified by G. E. Bucher, Research Institute, Bellevue, either did not "attract" ovipositing mosquitoes or did so only after a relatively long time. The same pools, however, were brought to the conditions found in phase (2) by inoculating them with 100 ml. of water from an "attractive" pool. They were then used by the mosquitoes for oviposition. Treated pools that had passed through the various phases of "attractiveness" could be returned to phase (2) by adding more capric acid. Evidently relative numbers of active bacteria in the pools determined the degree to which mosquitoes used them for oviposition, and this was coincident with the appearance of the water—brown and opaque with many actively reproducing bacteria as in phases (1) and (2) and later clear when the bacteria died and reproduction was minimal as in phase (3). Evidently bacteria responsible for the attractiveness were present in most of the pools, and capric acid acted as a fertilizer, supplying the carbon needed for their rapid growth.

Water taken from an "attractive" pool was clearly preferred to tap water in laboratory tests with other species of mosquitoes. Relative numbers of eggs or egg rafts laid in "attractive" compared with tap water were 1,190

and 71 for *Aedes aegypti* L.; 265 and 55 for *Culex pipiens* L.; and 24 and 4 for *Culex tarsalis* Cop.

Substances that can aggregate individuals of a field population at an appropriate stage should make a valuable contribution to control programmes, especially perhaps to those involving sterilization at a natural focus. Being non-persistent, relatively inexpensive and only briefly toxic to aquatic life (not necessarily a disadvantage in artificial pools), capric acid appears to hold great promise as such a substance.

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Increasing Frequency of the Typical Form of the Peppered Moth in Manchester

THE murk of nineteenth-century Manchester fostered the melanic form carbonaria of the peppered moth, *Biston betularia* (L.). The subsequent spread of this form, at the expense of the pale coloured typicals, throughout industrial regions in Britain and elsewhere in western Europe, is a striking example of rapid evolutionary response to a changing environment.

Records of *B. betularia* in Britain indicate that the incidence of the melanic form in industrial regions increased swiftly during the past 100 yr, but precise data on the rate of change are regrettably lacking¹. In Manchester the black form was prevalent by 1900, although typicals still occurred. The only large sample recorded from the area was taken between 1952 and 1964 at Didsbury, about 4 miles from the city centre^{1,2}. In all, 760 moths were collected, consisting of 749 carbonaria and eleven of the intermediate melanic form insularia, typicals being unrepresented.

The environment of the peppered moth is again changing. Smoke control, started in 1952, has led to a marked reduction in the amount of soot in the air. Old, buildings blackened by smoke are being cleaned, and new ones are being constructed of light coloured fabrics. To investigate whether these current changes are reflected by the population of *B. betularia* and of other polymorphic species of moths, between 1966 and 1969³ we collected the moths in mercury vapour light traps and assembling traps at sites between Prestwich to the north and Jodrell Bank some miles to the south of the conurbation. In all, 972 *B. betularia* were recorded of which twelve were insularia and twenty-five were typicals. By Fisher's factorial test the difference between the frequency of typicals in this sample and in the returns for 1952-64 is very highly significant.

The form insularia is variable in phenotypic expression, sometimes approaching the typical in appearance. In the Manchester area specimens tend to the darker rather than the paler end of the range of variation, so that in this region there is no danger of confusing the two forms. After correspondence with Mr H. N. Michaelis, the collector of the earlier material, and examination of some of his specimens in the Manchester Museum, we are convinced

that both sets of samples have been scored using the same criteria.

Some of the collecting sites at the southern end of the transect are quite rural in character, but the results are uniform over the entire length. Considering only moths in Didsbury and in Longsight, a more polluted locality two miles to the north, we obtained eight typicals and two insularia in 364 individuals. The frequency of typicals is again significantly different from the earlier one obtained from almost the same area ($P < 0.0001$), suggesting that there has been a recent resurgence of the typical form deep in the Manchester conurbation. Evidence of a small but significant increase in frequency of typical moths in the Liverpool area following the introduction of smoke control has been reported by Clarke and Sheppard⁴.

We thank colleagues in the Department of Zoology, Manchester University, for collecting samples.

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Synthesis of a Mucous Cuticle by a Zoanthid

MANY zoanthids (Coelenterata, Zoantharia) are known to produce a secretion which lies external to the epidermis¹. This structure is termed a "cuticle". Although it has been assumed that the cuticle is secreted by the epidermis, direct evidence of this is lacking. The source of substrates used in the synthesis of the cuticle is also unknown.

During studies on the movement of photosynthetic products of zooxanthellae to the tissues of their animal hosts, it was noticed that in *Zoanthus* sp., photosynthetically fixed ¹⁴C moved from the algae in the animal's gastroderm to the epidermis, and was then incorporated into the substance of the cuticle as it was secreted by the epidermis.

Specimens of *Zoanthus* sp. were collected at Punta Piñasco, Baja California, Mexico. They were maintained in seawater aquaria at 20°-23° C under constant aeration and daily illumination of about 1,000 foot candles. They were used in experiments within 5 days after collection.

The animals were incubated in the light and in the dark for 1 h in seawater to which NaH¹⁴CO₃ was added to give an initial activity of 10 µCi/ml. Specimens incubated in the light were then transferred to unlabelled sea water in the light. Specimens incubated in the dark were first placed in unlabelled seawater in the dark for 6 h before transfer to the light, to ensure that residual unincorporated H¹⁴CO₃ had been washed out from the tissues. 6 h after the end of the incubation, one dark-incubated and one light-incubated specimen were fixed in Bouin's solution. The dark-incubated specimens were then separately transferred to the light in fresh unlabelled seawater and all were exposed to daily illumination. At daily intervals for the first 10 days, and every 2 days subsequently for a total of 25 days, light and dark-incubated specimens were fixed. Specimens were prepared for autoradiography according to a method previously described^{2,3}. Paraffin embedded sections were cut at 5-7 µm, and tissues were exposed to Kodak NTB-2 liquid emulsion for 9 days and developed as suggested by the manufacturers.

Zooplankton was collected in coastal waters 33 to 35 m from Boothbay Harbor, Maine ($43^{\circ} 48' N$, $69^{\circ} 38' W$), simultaneously at the surface and 10 m and 30 m down, using 20 cm Clarke-Bumpus closing samplers hauled between 2 and 3 knots for 15 min. Tows were made at approximately hourly intervals from morning to evening on the pre-eclipse (control) and eclipse days. In the laboratory, the zooplankton was sorted into major taxa, and the numbers of each taxon per $10 m^3$ of water per sample were calculated. The time at mid-eclipse was 1348 EST (Fig. 1) when about 94 per cent of the Sun was obscured. At Boothbay Harbor the Sun was at an altitude of 34° ; sky clear with visibility about 15 km, wind 5 knots from the SW, and the sea calm. On the pre-eclipse day the sky was generally clear, but between 1300 and 1400 h a one-sixth cover of stratocumulus clouds passed over the sampling area; sea conditions were calm throughout the day.

There were nineteen taxa in the samples, five of them in abundance, accounting for over 90 per cent of the plankton—copepods, decapod larvae, chaetognaths, cirriped larvae and gastropod eggs. Copepods were dominant, constituting 70 per cent of the total. Of the sixteen species in the samples only two were numerous—*Pseudocalanus minutus* and *Calanus finmarchicus* (more than 85 per cent of the total copepod species).

The vertical distributions of the zooplankton differed significantly (Figs. 2 and 3). Adult chaetognaths (*Sagitta elegans*) were concentrated near the bottom on both days although numbers in the upper 10 m increased slightly in the evening. Larval decapods were concentrated at 10 m during daylight and moved to the surface after sunset (about 1756 h EST) on both days. The vertical distribution of larval cirripeds was more variable. They were most numerous 10 m deep during daylight; notable exceptions occurred during the eclipse, when cirripeds were concentrated at 30 m between 1100 and 1230 h, and again after sunset, when they were abundant over the bottom. The non-motile gastropod eggs were widely distributed among the depths sampled on both days.

Copepod distributions were also variable; concentrations increased at the surface on March 7 during the mid-

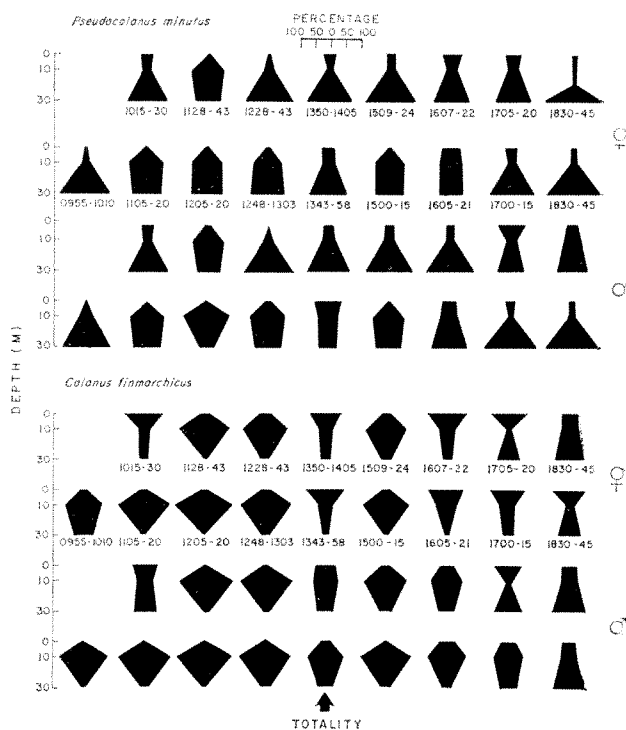


Fig. 3. Percentage occurrence of male and female *Pseudocalanus minutus* and *Calanus finmarchicus* at three depths (0, 10, and 30 m) on the pre-eclipse and eclipse days.

eclipse period from the pre-totality concentrations at 10 m followed by a return to 10 m after totality. In the late afternoon when light conditions approached the level of totality, copepods moved to the surface and were concentrated at the bottom after sunset. On the day before the eclipse *P. minutus* was generally concentrated near the bottom during daylight. Both sexes rose to the surface in late afternoon (between 1600 and 1700 h), and moved to 30 m after sunset (1756 h). The notable difference was the concentration at 10 m between 1100 and 1200 h. This early morning change in depth distribution also occurred on the eclipse day for both sexes; *P. minutus* then remained concentrated in the lower two-thirds of the water column until mid-eclipse, when numbers increased at the surface. They left the surface in the hour following totality, rose again to the surface in the late afternoon, and were concentrated at 30 m after sunset. Males responded more actively at totality than females.

By contrast, *C. finmarchicus* was higher in the water column on the morning of the day before the eclipse, but was otherwise concentrated 10 m deep from mid-morning to about 1300 h on both days. During the mid-eclipse period between 1300 and 1400 h on the pre-eclipse and eclipse days, this species moved to the surface; in the next hour, both sexes concentrated at 10 m, followed by a movement toward the surface in the late afternoon (1600 h), and a subsequent downward movement in the evening. The upward movement of females was greater than males at 1300 h and in the late afternoon of both days (Fig. 3).

During sampling, temperatures were nearly homogeneous throughout the water column. The temperature difference was only 0.4° ranging from 1.6° at the surface to $2.0^{\circ} C$ on the bottom. Salinities ranging from 31.38 parts per thousand at the surface to 31.81 on the bottom, were similar for both days, indicating that changes in the composition of zooplankton from large scale advection were minimal.

Among the zooplankton in our samples, *P. minutus* elicited the response that was synchronized most closely with the exogenous changes of light during the eclipse. It had a vertical distribution similar to the classic descrip-

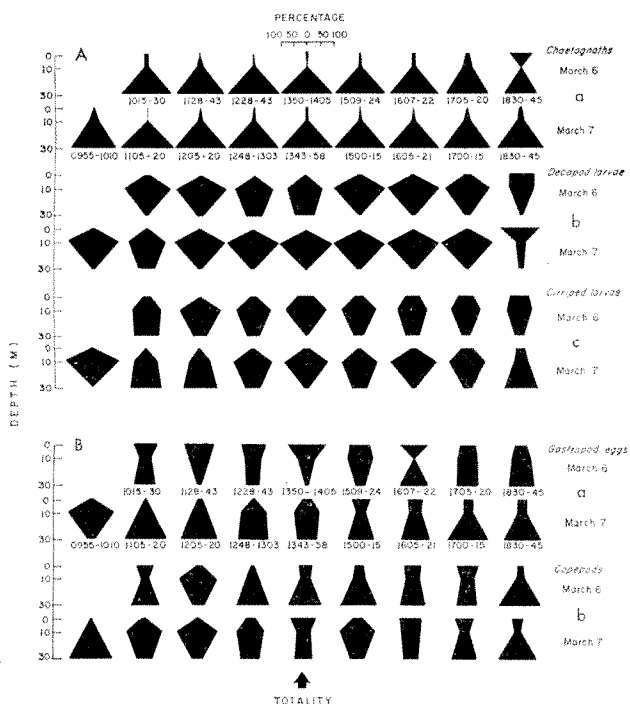


Fig. 2. A, Percentage occurrence of chaetognaths (a); decapod larvae (b), and cirriped larvae (c) at three sampling depths (0, 10, and 30 m) on the pre-eclipse and eclipse days. B, Percentage occurrence of gastropod eggs (a), and copepods (b) at three depths (0, 10, and 30 m) on the pre-eclipse and eclipse days.

tion of the diurnal migration of crustacean plankton by Bainbridge², but on a shorter time scale. Changes in the vertical distribution of the other zooplankton revealed varying degrees of threshold response to light changes. The threshold of *C. finmarchicus* was the lowest, responding to changes in light intensity within a 1,000 foot candle range (from 5,500 to 6,500 foot candles) on the pre-eclipse day at a time period (about 1340 h) coincident to a 6,000 foot candle decrease in light during totality (Fig. 1). An alternative explanation for the coincident occurrence of *C. finmarchicus* at the surface can be found in the results of Harris⁹, who observed an endogenous rhythm of vertical distribution of the cogenetic species, *C. helogolandicus*. The similarity in the upward movement of *Calanus* to the surface at dusk on both days and the subsequent distribution toward bottom after sunset support our argument for the observed differences in the vertical movements of *Calanus* as an exogenous response rather than an endogenous rhythm. *Calanus* can move in short bursts of about 66 m/h (2 min) and at 15 m/h during a longer time (1 h) (ref. 10).

Among the other taxa, chaetognaths showed little change in vertical distribution. Decapod larvae did not respond to light changes of the eclipse, but did move up in the water column after sunset. Distribution of larval cirripeds was more variable; although they did not respond to the eclipse, this group is known to be phototactic¹¹, and appeared to be maintaining themselves at 10 m for a sustained period during daylight.

Our observations on the movements of *P. minutus* are in agreement with the results of Skud made during the solar eclipse of July 20, 1963. He found that *P. minutus* moved toward the source of light change during totality. The increased response of female *C. finmarchicus* over males to light changes was also reported by Clarke¹² during his studies of diurnal vertical migration in the Gulf of Maine. The strong responses reported by Petipa of chaetognaths and decapod larvae to light changes during the eclipse of June 30, 1954, in Sevastopol Bay differed from our results. This can be attributed to differences in the environmental conditions and species composition of the zooplankton.

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Spatial Distribution and Pattern of Some Atlantic Reef Corals

THE zonation of stony corals on both Atlantic and Pacific coral reefs has been well documented. Descriptions of coral reefs, wherein communities or associations of species are developed into zones or bands in relation to water depth, wave action and distance from shore, have been published for the Caribbean¹⁻⁶, and for Pacific reefs⁷⁻¹¹.



Fig. 1. Aggregation of colonies of the coral *Favia fragum* on the surface of a reef with a white marker 2.5 inches in diameter.

Pattern and horizontal spatial distribution of corals within the respective zones have received little attention¹², although certain species have been reported as dominant or exclusive within certain zones^{1,3,4,8}.

A single zone within the fringing reef complex around Barbados, West Indies, was selected for investigation by the method of random quadrat analysis^{13,14}. The surface of the reef crest zone has been previously described² and is composed of dead coral rock which is secondarily encrusted by several species of coral. The surface seems to be homogeneous with respect to water depth, wave action, temperature, salinity and surface topography. Four species of coral occur commonly on all the reefs examined: *Favia fragum* (Esper), *Porites porites* (Pallas), *Porites astreoides* Lamarek and *Agaricia agaricites* (Pallas).

Eighty quadrats, each 1 m square, were located at random on a uniform area of reef. Colonies of each of the four species of corals were counted on each square metre, and on each 1/10 square metre for *Favia* and *Agaricia*, for each quadrat. Mean numbers of colonies per quadrat were calculated together with the ratio of variance to mean for each species. Results are shown in Table 1.

Table 1. RATIOS OF VARIANCE TO MEAN OF CORAL COLONIES FOR RANDOM QUADRATS IN THE REEF CREST ZONE

Quadrat size	Species			
	<i>Favia</i>	<i>Agaricia</i>	<i>P. porites</i>	<i>P. astreoides</i>
m ²	312/30	407/34	5/2	59/14
1/10 m ²	16/4	13/5	—	—

The high ratios of variance to mean in three species are a clear indication of non-random distribution¹³ and are evidence of aggregated or contagious distribution of corals on the reef crest zone. A clump of thirteen colonies of *Favia fragum* is shown in Fig. 1.

Because the environmental factors which may influence the distribution of corals are apparently the same throughout the reef crest zone, the causes of the contagious distribution are likely to be biological in nature. The reason for the non-random distribution of corals in terms of larval settlement, growth and survival in a number of reef zones are currently under investigation.

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Strontium-Isotope Evidence for Marine or Freshwater Origin of Fossil Dipnoans and Arthrodires

STRONTIUM has a uniform isotopic composition in well mixed regions of the sea^{1,2} because of its long residence time. Though the strontium isotope composition of seawater has fluctuated through the Phanerozoic³, it apparently has been relatively homogeneous during particular periods of time. Marine organisms that utilize calcium (and strontium) in their shells or skeletons are known to incorporate strontium of marine isotopic composition. Strontium in fresh or brackish water may diverge isotopically from the marine value, depending principally on the age and Rb/Sr ratio of source rocks that contribute soluble strontium⁴. Thus the isotopic composition of strontium in fossil aquatic organisms may rule out a marine origin for the organisms, provided that the age of the organisms is known, and the isotopic composition of the fossil has not been perturbed by strontium addition or exchange; on the other hand, the isotopic data may be consistent with (but not prove) a marine origin. Non-equilibrium of strontium in phosphate and strontium in carbonate (or seawater) during diagenesis may be inferred from a lack of microscopically detectable alteration, and by analogy with the non-equilibrium of certain clay-carbonate systems⁵.

A vexing biological problem has been the marine or freshwater habitat of many early and middle Palaeozoic fishes^{6,7}. In addition to the difficulty in distinguishing sediments of freshwater, marine and brackish environments, fishes found preserved in rocks of undoubted marine origin may not have been entirely marine in habit; they may have been freshwater types transported by rivers into the sea either alive or as cadavers, or they may have been euryhaline, returning to the rivers to spawn. The Dipnoi (lungfishes) have a special interest in this connexion. For various physiological reasons and because it seems reasonable to believe that "lungs in fishes are an adaptation for life in poorly oxygenated waters . . . such as are unlikely in the sea . . ."⁸, the Dipnoi have been considered by most zoologists to be of fresh-water origin. The earliest known lungfish are, however, *Dipnorhynchus* from the marine Hunsrück-schiefer of Germany and Taemas Group of New South Wales, and *Uranolophus* from the "marginal marine" Beartooth Butte Formation of Wyoming. This evidence has been used to support a marine origin for dipnoans.

At Wee Jasper, New South Wales, *Dipnorhynchus* (or a close relative) occurs in the upper Emsian or lower Eifelian (Lower-Middle Devonian) *Receptaculites* Limestone of the Taemas Group in association with numerous arthrodires (Placodermi), particularly *Buchanosteus*. The invertebrates in these limestones clearly indicate a marine environment, and there is strong independent stratigraphic evidence that the fish were not washed in⁹. Further, it is generally agreed that though the arthrodires

probably originated in freshwater, at least some of them had adapted to marine conditions before the end of the early Devonian. Hence it is not surprising to find the group well represented at this locality.

Samples of *Buchanosteus* and *Dipnorhynchus* and associated carbonate from Wee Jasper were collected for analysis. The sedimentary rocks and fossils show no sign of metamorphism or other alteration, either in the field or in thin section. The delicate structure of the bone is well preserved in the rock; the few complete skulls that have been found also show little deformation.

Representative phosphate-free samples of carbonate were dissolved in 1 N HCl and strontium for isotopic analysis was obtained from the detritus-free supernate. Insoluble residues amounted to less than 2 per cent of the samples. Phosphate from the two fish was cleaned of carbonate by leaching in 1 N HCl. X-ray diffraction of the phosphate shows lines which are largely apatite or a related phosphate. No evidence of carbonate, or detritus such as feldspar or clay, was detected in the analysed phosphate samples. Amounts of rubidium and strontium were determined on whole-rock carbonate and on the carbonate-free phosphate by X-ray fluorescence. Strontium was separated from the samples by dissolution and standard ion exchange techniques and isotope composition was determined with a 6-inch, Nier-type mass spectrometer. Results are listed in Table 1. Because of the low Rb/Sr ratios and the moderate age of the strata, measured ⁸⁷Sr/⁸⁶Sr ratios are essentially initial ratios, or ratios at time of deposition.

Table 1. STRONTIUM ISOTOPE DETERMINATIONS

Sample	Rb p.p.m.	Sr p.p.m.	Rb/Sr	⁸⁷ Sr/ ⁸⁶ Sr	
Carbonate	10.2	1370	0.007	0.7086	±0.0004
Phosphate (<i>Buchanosteus</i>)	<1.0	760	<0.001	0.7088	±0.0004
Phosphate (<i>Dipnorhynchus</i>)	<1.0	600	<0.002	0.7090	±0.0007

The ⁸⁷Sr/⁸⁶Sr ratios are identical within the analytical uncertainties, and are consistent with the view that the skeletons of both types of fish were formed while the fish inhabited the sea—that is, they were marine or euryhaline.

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Thermoluminescence of Biological Materials

THERMOLUMINESCENCE may be observed from many chemical compounds, both in crystalline and in amorphous form. The compounds so far investigated have usually been mineral or synthetic materials, but there is no reason to suppose that the various salts deposited in biological materials have a structure excluding this property.

The bones of vertebrates, in which the mineral salts are deposited as an intercellular substance are one of the best examples. Dr Z. Jaworowski had at his disposal radioactive bones of dinosaurs¹, and we started off our

investigation with these bones. Later, the measurements were extended to other organic fossil and contemporary samples.

Our chief difficulty resulted from the change of the sample associated with the combustion of organic compounds during the heating process for obtaining a glow curve. The organic compounds should therefore be removed from the sample before the measurement, and

All of the twenty-four different samples investigated showed radiothermoluminescence. In the case of sample D-XV, the natural radiothermoluminescence was so strong that it was visible with the naked, well adapted eye. Some samples also showed a pronounced tribothermoluminescence, equivalent to a dose of many thousands rads. Figs. 1 and 2 show some examples of the glow curves obtained.

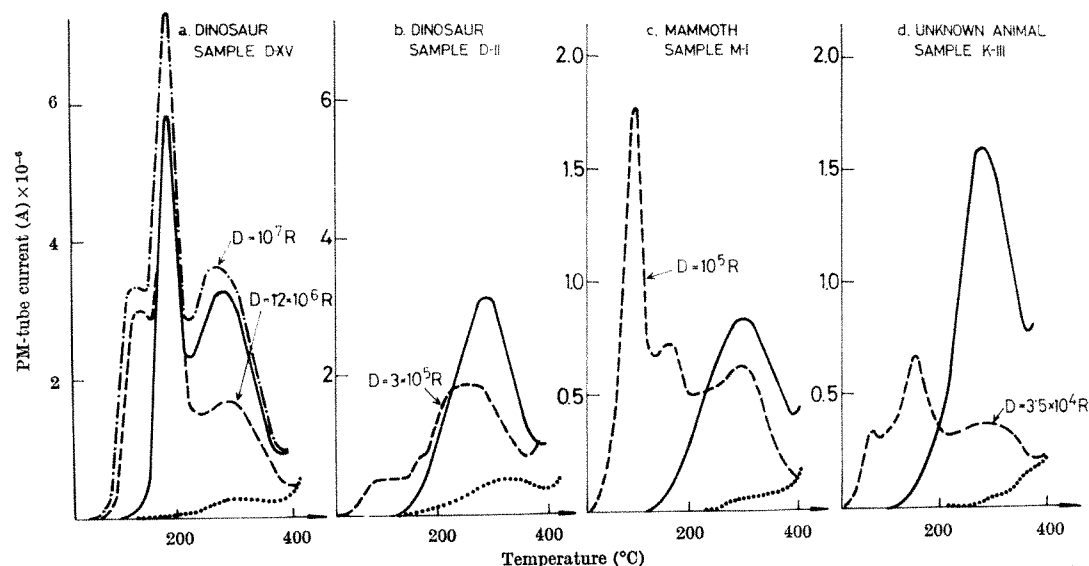


Fig. 1. Glow curves of fossil bones. —, Natural thermoluminescence; ---, radiothermoluminescence after artificial irradiation; ..., tribothermoluminescence.

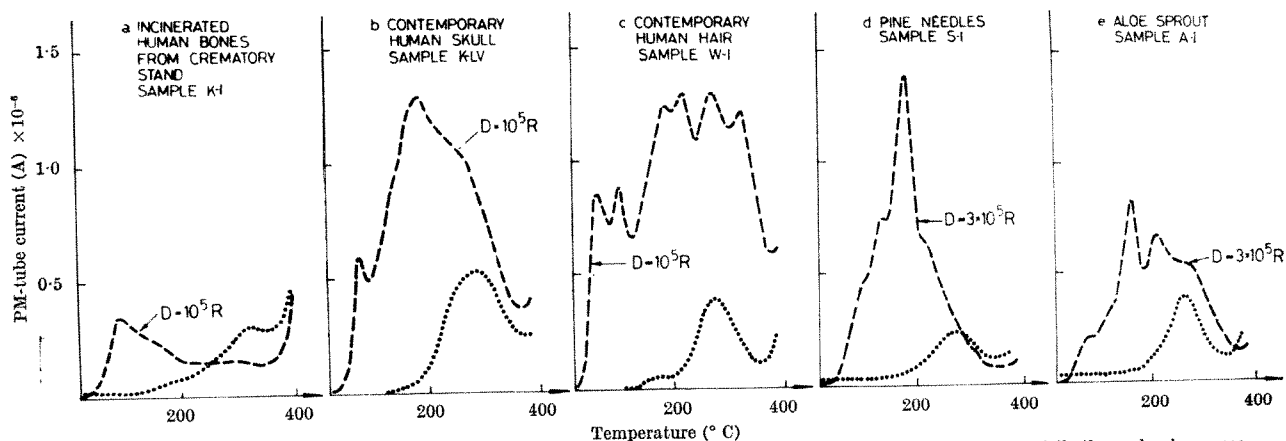


Fig. 2. Glow curves of incinerated organic samples. ---, Radiothermoluminescence after artificial irradiation; ..., tribothermoluminescence.

it is advisable to make it without any great temperature increase, because only then is the natural radiothermoluminescence preserved. During a very long period underground, the fossil bones have absorbed a high radiation dose from the radionuclides contained inside deposits and from cosmic radiation. At the same time, natural processes removed organic compounds from the fossil bones. The other samples investigated were simply incinerated at 400° C before the thermoluminescence measurement because no better preparation method has as yet been developed. For this reason they were examined for the artificially induced radiothermoluminescence only.

The glow curves were registered by an *xy* recorder controlled by the photomultiplier tube current. A blue glass filter was interposed between the photocathode and the 10 mg sample, which was heated by a Kanthal strip at 100° C/min in an air atmosphere². Artificial irradiations were administered with cobalt-60 gamma sources. Tribothermoluminescence was induced by a vigorous rubbing of the sample in a mortar.

The first results of these measurements seem to be very interesting. Work has therefore been started on a more detailed investigation of fossil bone radiothermoluminescence. It is possible that the radioluminescence may be used for their dating, similar to the dating of ancient pottery^{3,4}.

We thank Dr Z. Jaworowski from the Institute for Nuclear Research, Warsaw, for his suggestions and for supplying the samples of dinosaur bones.

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Agar Culture Medium modified to approximate Soil Conditions

SMALL soil or mud surface plants frequently fail to develop their characteristic morphology when grown on an ordinary agar medium. In particular this applies to the parts penetrating the substrate. Inspection frequently suggests that what is wrong is that agar, unlike soil, is a medium which does not exclude light as a formative factor. We have devised a simple corrective system with good results.

The plants tested were the green alga *Fritschiella tuberosa* (inoculum a small agar disc cut from a stock culture, derived from material from Allahabad, India, sent by Dr A. K. Mitra) and the moss *Funaria hygrometrica* (inoculum four spores per dish, from a local weed population). The medium consisted of 125 ml. of mineral agar (10 g/l. any proven formulation will do; we used that cited in ref. 1) per 9 cm diameter, 5 cm high Pyrex crystallizing dish with Petri dish lid. After inoculation the agar surface was overlaid with a sterilized heavy aqueous suspension of powdered charcoal applied with a pipette, to yield a settled charcoal layer 1–2 mm deep. Illumination was restricted to the surface layer by attaching black paper to the side and bottom of the dish (A). Two kinds of controls were cultured concurrently, one with charcoal but no black paper (B), the other with unobscured agar (C). Incubation was at 17° C with 40 W cool white fluorescent bulbs providing 325 foot candles as illumination; the photoperiod was 16 h day and 8 h night.

Development of *Fritschiella* was most rapid in the unobscured control dishes (C), forming prostrate and tufted erect green filaments, and abundant others which grew down into the agar. The latter were mostly also fully green, some were pale and unsharply rhizoidal. In all the green filaments there was a random and messy alternation of uniseriate and parenchymatous areas, the parenchymatization starting at the surface of the agar. Typical *Fritschiella* differentiation was not achieved. In the test cultures proper (A) erect *Stigeoclonium*-type filaments developed and narrow colourless rhizoids penetrated the agar. Parenchyma soon began to form, but only in the upper part of the carbon film, yielding a stratum of almost colourless tubers. The resultant thalli had the diagnostic characters of *Fritschiella*, and closely matched field material grown on mud. Only in old cultures did additional parenchyma form in the sub-aerial filaments. In the controls with carbon but no black paper (B) there was, even at the centre, a more limited production of tubers, and, instead of only colourless rhizoids, a heavy growth of ultimately largely parenchymatous green filaments down into the agar.

In the test culture of *Funaria* (A), there was a reasonably sharp caulonema (developed at or just below the agar surface)—chloronema differentiation, and a lush development of totally colourless and richly branched rhizoids penetrating the agar, as in material grown in the field. The rhizoids from the resultant gametophores were also free of chlorophyll; the only brown wall colour in rhizoids was in gametophoric ones above the carbon only. The unobscured control (C) had a lesser degree of differentiation; the scarce and sparsely branched rhizoids had small green plastids and there was a heavy development of green filaments into the agar, yielding gametophores even below the agar surface. Rhizoidal differentiation in the control with carbon but no black paper (B) was better in the centre of the dishes, but again all the agar contained green filaments. (A newly observed culture artefact is that here, and in the low light intensity of the centre of the dish only, the coarse basal gametophoric rhizoids, once below the carbon, not only turned green but continued to grow as green filaments. By contrast, in control (C), the equivalent structures, apart from their downward growth, started essentially like

caulonema but at greater depth lost their green colour.)

Clearly agar must be totally obscured in this type of culture. Only then is differentiation fully normal and growth in the agar restricted to colourless rhizoids. The carbon film method is messy in the sense that for examination the material has to be slaked in ample water. Making the entire agar medium black may possibly suffice in the case of *Funaria*. But to find out which environmental factors induced "normal" differentiation will require much further experimentation. While it seems that for certain of the phenomena light exclusion alone may be responsible, there is a strong suggestion that for others the texture of the coating (gas exchange?, adsorption?) may also be involved. Thus *Fritschiella* tubers are formed in the upper portion of the carbon film only, and apparently not in utter darkness; it seems that they are initially practically colourless, but gradually become faintly green. At full maturity they also contain some carotenoid pigments, but far less than the exposed parts of the plant, which turn bright orange.

There is an extensive literature about the differentiation of moss protonema, and many of the results are doubtful and inconclusive. Much of the work requires repetition, using a dark substrate so that differentiation is normal.

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Algae as Nutrient Material for studying Ca-Sr Relationships in Heterotrophic Organisms

A RECENT review of the mineral nutrition of algae¹ summarizes the inorganic requirements and substitution elements for a number of green algal forms. Among those presented, it was especially noted that algae which can grow with an Sr replacement for Ca, such as *Chlorella*² or *Protosiphon*³, constitute a potential tool for investigating Ca nutrition in heterotrophic organisms. They may be particularly useful for comparing the effects of an adequate Ca supply with the effects of making Ca deficient and replacing it with Sr.

The feasibility of such a use for the alga, however, depends on several suppositions. The first is that the alga will provide all the organic nutrients needed by the heterotroph; second, that the alga in Sr, except for its lack of Ca, would be as suitable a source of nutrients for the heterotroph as the same alga grown in adequate Ca; third, the alga must be able to grow in Sr with little enough Ca to make it Ca-deficient in relation to the heterotroph. In order to test these suppositions, autoclaved extracts of *Protosiphon botryoides*, a unicellular green alga, were used as nutrient material for the heterotrophic protozoan *Tetrahymena pyriformis*. The selection of these two organisms seemed ideal for studying the interrelationships of Ca and Sr; *Protosiphon* can synthesize organic materials starting with CO₂, water and inorganic salts using³ either Ca or Sr, and *Tetrahymena* has, for the most part, defined organic and inorganic requirements that simulate those of higher heterotrophs⁴.

In our experiments *Tetrahymena* used for inoculum were removed from stock medium, washed to remove Ca and other nutrients, and placed in test media prepared from algal material. Basically, two types of test medium were prepared. 1 g (dry weight) of *Protosiphon* grown in mineral nutrient medium⁵ containing Ca was autoclaved in fresh mineral medium. The extract obtained from this

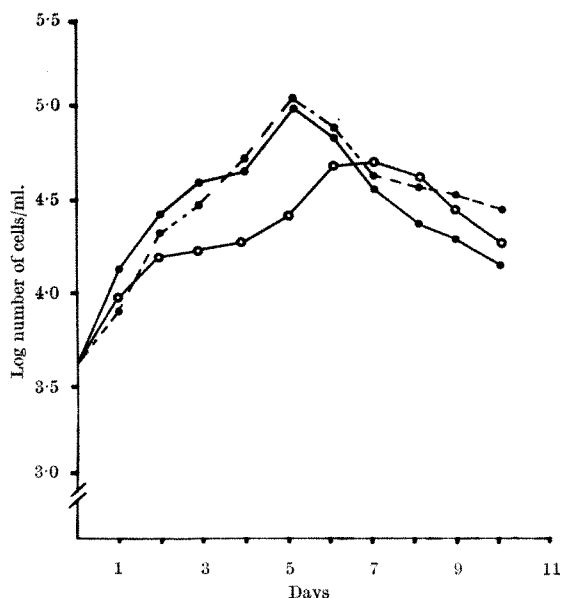


Fig. 1. Growth rates of *Tetrahymena pyriformis* in Sr-grown alga (○—○), in Ca-grown alga (●—●), and in Sr-grown alga enriched with Ca (●—●—●).

was filtered, rendered axenic and finally inoculated with *Tetrahymena*. The second type of test medium was made from 1g (dry weight) of *Protosiphon* grown in mineral medium containing no Ca but an equimolar Sr replacement. The dry Sr alga was placed in fresh Sr replacement mineral medium and autoclaved. Again, the extract obtained was filtered, made axenic and inoculated with *Tetrahymena*. A final variation involved adding inorganic Ca before inoculation with *Tetrahymena*.

With regard to the first supposition, organic nutrient fractions of algae, as percentage of total dry weight, differ considerably according to cultural conditions⁶. If the cells are young and growing vigorously, protein content will be relatively high and carbohydrate low, while older cells increase in carbohydrates and lipids as the growth rate of the culture decreases. Actively growing algal cells that have adequate Ca are known to produce the organic materials required by some heterotrophs⁶. A comparison of nutrient materials of *Protosiphon* grown in Ca medium and Sr medium is given in Table 1. The similarity in composition of both kinds of *Protosiphon* suggests that either *Protosiphon* extract, Ca or Sr can supply the organic requirements of heterotrophs, including *Tetrahymena*. In this event, a heterotroph with a Ca requirement replaceable by Sr could be sustained by extracts of *Protosiphon* grown in Sr rather than in Ca. If, on the other hand, Ca was not replaceable by Sr in the heterotroph, *Protosiphon* would not sustain it.

Table 1. NUTRIENT COMPOSITION OF *Protosiphon botryoides* CELLS IN Ca-MEDIUM OR IN Sr-MEDIUM DURING THE LOGARITHMIC GROWTH PHASE

Nutrient	Pet cent total dry weight of cell, Cells from Ca-medium	Cells from Sr-medium
Crude protein	47.00	47.00
Lipids	37.00	38.00
Minerals		
Mn	6.30×10^{-3}	6.32×10^{-3}
P (as PO_4)	5.01	3.99
Mg	1.39	1.08
Fe	3.31×10^{-2}	3.96×10^{-2}
Ca	2.30×10^{-3}	1.59×10^{-4} *
Total	6.51	5.17
Carbohydrates		
Free reducing sugar	4.50×10^{-2}	4.90×10^{-2}
Sucrose	2.50×10^{-2}	3.50×10^{-2}
Oligosaccharide	5.32×10^{-2}	7.00×10^{-2}
Starch	2.52	2.27
Hemicellulose	5.78	6.30
Cellulose	0.74	0.74
Total	9.64	10.09

* By neutron activation analysis carried out by Nuclear Science and Engineering Corporation, Pittsburgh, Pennsylvania.

The second supposition can be tested by adding inorganic Ca to Sr-*Protosiphon* extract. If the organic composition of the Sr-grown alga is as suitable as Ca alga, the growth rate of *Tetrahymena* in the Sr algal material enriched with Ca should be as good as in Ca-grown algal material. This is what indeed happened, as Fig. 1 shows.

Contamination, which is the concern of the third supposition, has to be considered in any essential element study. The Sr alga has better possibilities for lowering Ca contamination than alternative methods for producing food, deficient in Ca, for heterotrophs, because by this procedure, purification to remove Ca involves water and inorganic materials but not organic products of natural origin, which are more difficult to free of Ca. The amount of contaminant Ca in the Sr *Protosiphon* cells used has been determined by neutron activation analysis to be less than $1.46 \mu\text{g/g}$ (dry weight). The Sr salt used in the liquid mineral medium was found to contain Ca at $20 \mu\text{g/g}$. With contamination of this order, calculations indicate that the Sr algal extracts on which the *Tetrahymena* grew contained $1.5 \mu\text{g/l}$. Ca or less.

Tetrahymena that had been washed carefully before use as inoculum multiplied through three subcultures in the Ca algal extract and also through the same number in the Sr algal extract. The successive subculturing involved the production of about eighteen generations of *Tetrahymena*; thus any growth factor or mineral introduced within a *Tetrahymena* cell used as inoculum would be expected to be diluted by the eighteenth generation by a factor of approximately $1/120,000$ and should have become growth-limiting by this time if not supplied also by the algal extract. While there has been some difficulty in demonstrating a Ca requirement in *Tetrahymena*⁷, it is generally considered⁴ to require Ca. The inoculum-borne Ca could not be expected to be adequate to meet this requirement throughout the subcultures in Sr algal extract medium.

Growth in each of the two algal extract media, Ca and Sr, is compared, along with growth in stock medium, in Fig. 2. Neither algal extract medium used supports growth as well as the stock medium. In Sr, the maximal number of organisms produced and the overall growth rate is lower than in Ca. This indicates that some or all of the metabolic processes requiring Ca occur less efficiently

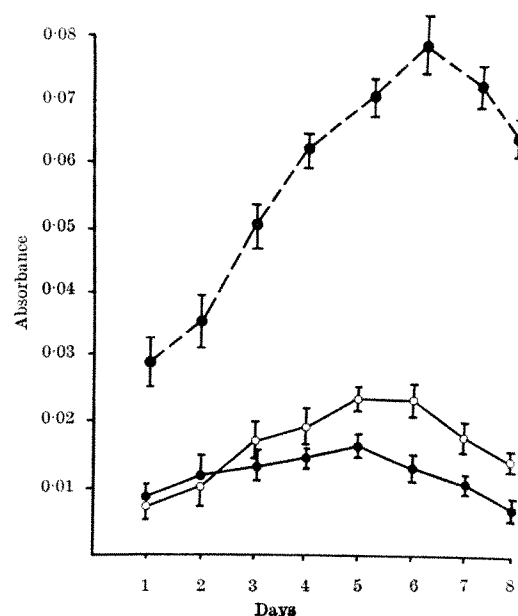


Fig. 2. Variations in the growth of *Tetrahymena pyriformis* in peptone medium (●—●—●) and in algal extract medium containing calcium (○—○) or in algal extract medium containing strontium (●—●). The standard error is shown for each mean. Each curve represents eight replicate measurements.

when only Sr is available. The continued growth of *Tetrahymena* in the Sr algal extract medium, however, indicates that Sr may substitute for Ca metabolically, as it is known to do in several fungi and algae, or that the small amount of Ca present as contamination may be adequate for the growth in the Sr medium.

This relationship between *Protosiphon* and *Tetrahymena* is one example of the way in which the contents of algal cells can be modified to produce a particular test diet for a heterotrophic microorganism. There should be other similar ways of using algae for studying mineral nutrition of heterotrophic organisms.

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Assay of Algal Nitrogen Fixation in the Marine Subtropics by Acetylene Reduction

WHILE SCUBA diving in the Tongue of the Ocean (Bahamas) and off the coast of south-eastern Florida near Miami, we found extensive benthic growths of blue-green algae, which are especially prolific between depths of 20 and 50 m, and considerably more abundant in summer than winter. At their peak, the algae produce continuous, and sometimes almost unialgal felts at the surface of calcareous sediments between and beyond the limit of the coral heads of the offshore reef. During the winter they persist in isolated patches in sheltered positions beneath overhanging coral.

The felts are characteristically red to brown in colour, because chlorophyll is masked by phycobilins, and are heavily dominated by members of the Oscillatoriaceae. Using Drouet's taxonomic scheme¹, we identified *Schizothrix calcicola*, *S. mexicana*, *S. tenerrima*, *Porphrysiphon notarisii*, *P. kurzii*, *Microcoleus lyngbyaceus* and *Spirulina subsalsa*. An unidentified species of *Calothrix* was found in one sample from the Tongue of the Ocean, and on one occasion some filaments of an *Anabaena* were recovered 30 m down at the edge of a reef off Miami. Within the range of depths examined, however, heterocystous forms seem to be rare.

Wyatt and Silvey's² work on *Gloeocapsa* sp. in pure culture has shown that lack of heterocysts does not necessarily preclude nitrogen fixation, and so we investigated this activity in the communities we found. We used the acetylene-reduction technique described by Stewart, Fitzgerald and Burris³, based on earlier observations^{4,5}. Samples of gas (up to 250 μ l.) were analysed by gas chromatography using a dual- H_2 flame ionization detector (Varian Aerograph Co., California) and a column of

'Poropak R' (100–120 mesh) packed in a stainless steel column (10 feet \times 1/8 inch bore). The columns were operated at 75° C with a flow of carrier N_2 of 25 ml./min. Retention times were 3.3 min for C_2H_4 , and 4.5 min for C_2H_2 . Acetylene reduction by algae was assayed in 6.5 ml. serum bottles sealed with serum caps. The gas phase occupied 5 ml. (only approximate for assays carried out in the ocean) and was composed of 2 or 10 per cent C_2H_2 in an artificial air mixture (20 per cent oxygen; 0.04 per cent CO_2 with a residue of argon) or argon. A nitrogen-fixing marine *Nostoc* sp. (supplied by Dr C. B. van Baalen), which was the control organism, was grown and incubated in nitrogen-free ASP-2 medium⁶. We found that it was unsatisfactory to return samples of algae to the laboratory for assay because of gross leaching of phycobilins during transit and this could not be prevented. Attempts were then made to perform the assays *in situ* using SCUBA. The artificial air mixture used initially proved inhibitory to acetylene reduction by the *Nostoc* sp. (Table 1) presumably because of the increased partial pressure of oxygen, in the gas mixture, on the seafloor. Increased pressures of oxygen are known to have inhibitory effects on photosynthesis and nitrogen fixation^{7,8}.

Table 1. ACETYLENE REDUCTION BY A MARINE *Nostoc* SP. AT SEA-LEVEL AND ON THE SEABED

Experiment No.	Depth (feet)	C_2H_2 formed (nmoles/h/mg of protein)	
		A	B
1	0	11.5	9.8
	90	11.7	6.6
2	0	16.0	8.5
	225	8.4	0.0

These results are from two separate experiments. A, Argon+acetylene; B, artificial air+acetylene. In experiment 1, the acetylene concentration was 2 per cent but in experiment 2 it was raised to 10 per cent for assays conducted in the laboratory (sea-level), which were carried out at 30° C and illumination of 500 foot candles. The bottles contained 0.3–0.6 mg of protein in experiments 1 and 2, and 4 mg of protein in experiment 2. Protein was determined by the method of Lowry *et al.*⁹ after hydrolysis of the alga with NaOH.

A mixture of 2 per cent acetylene in argon was then used for assays on the seabed. Samples of gas were transported to the site in disposable polypropylene hypodermic syringes fitted with gas-tight stopcocks and hypodermic needles (gauge No. 18). The syringes were clamped tightly on a lead-weighted carrying frame. *Nostoc* was transported in serum bottles filled with nitrogen-free ASP-2 medium and the gas was injected through the cap after insertion of a second needle to allow excess medium to escape. For the assay with indigenous blue-green algae, serum bottles were transported to the seafloor, at depths down to 80 m, with needles inserted in the caps. On arrival caps, with needles attached, were removed and algal material was transferred to the bottles. The serum caps and needles were replaced and the gas mixture was injected into the bottles, now filled with seawater. An hour later, the gases were drawn back into the syringes and returned to the laboratory for chromatographic analysis.

While *Nostoc* controls *in situ* always produced ethylene from acetylene (Table 1), parallel trials with the benthic cyanophyte flora were consistently negative. Laboratory tests with *Calothrix* sp. from the Tongue of the Ocean and with *Anabaena* sp. from the reef off Miami, however, have given positive indications of a capacity for nitrogen fixation. Furthermore, and in contrast to the benthic algae, tests with planktonic *Trichodesmium* sp. (*Oscillatoria erythraea*), collected in the Florida Current, have shown clear evidence of light-dependent acetylene reduction (34.3 nmoles/h/mg of protein). It should be noted that this material was largely free of other micro-algae and that direct phase contrast microscopy disclosed scarcely any contaminating bacteria. This finding supports earlier evidence for nitrogen fixation possibly attributable to *Trichodesmium* gathered by Dugdale, Menzel and Ryther¹⁰ who used isotopic nitrogen.

This work was supported by a grant from the US National Science Foundation. We thank Mr W. T. Mount for assistance in the field.

Addendum: Since this communication was accepted for publication, isolated colonies of *Anabaena* sp. were found at depths of 15 m off the coast of south-east Florida. This material gave strongly positive results for acetylene reduction with the *in situ* assay we have described.

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Possible Evolutionary Significance of Polyunsaturated Fatty Acids in Blue-Green Algae

AMONG eukaryotes, α -linolenic acid (18:3 α) and certain related polyunsaturated fatty acids occur as major fatty acids only in photosynthetic organisms¹, where they are concentrated in chloroplasts as components of the acyl lipids². Photosynthetic bacteria, by contrast contain saturated and monounsaturated fatty acids exclusively, a character which they share with nearly all non-photosynthetic bacteria³⁻⁵. Blue-green algae are the only prokaryotes which photosynthesize as do green plants, and their fatty acid composition is thus of particular interest from the evolutionary standpoint. Many filamentous blue-green algae have been shown to contain polyunsaturated fatty acids^{6,7,8}, but these compounds are absent from three unicellular blue-green algae so far examined: *Anacystis nidulans*, *Anacystis marina* and *Synechococcus cedrorum*^{6,7,9}. This has prompted us to undertake a more extensive survey of the fatty acid composition of blue-green algae, with particular attention to unicellular representatives.

The biological material consisted of a large collection of bacteria-free (axenic) strains accumulated in this laboratory during the past 5 yr. Most of these were isolated locally, but a few were obtained from other laboratories. We have found it impossible to identify most of these strains taxonomically by the criteria now generally used for the classification of blue-green algae so that, for the purposes of the present discussion, we have assigned the strains to a series of typological groups, each readily and unambiguously distinguishable in terms of a few structural properties (Table 1). Each typological group is identified by an applicable generic name, and possible alternative generic assignments for the strains included are indicated in footnotes. Every strain in each typological group is an isolate of independent origin. In many cases, strains assigned to a single typological group are readily differentiable from one another. Accordingly, typological groups that comprise several strains probably include representatives of several different species.

Table 1. KEY TO THE TYPOLOGICAL GROUPS RECOGNIZABLE AMONG THE BLUE-GREEN ALGAE EXAMINED

	No. studied
(A) Filamentous	
(1) Produce heterocysts when grown with a limiting supply of combined nitrogen	
(a) Heterocysts basal, trichome tapering from base to apex. <i>Calothrix</i> type	1
(b) Heterocysts intercalary, trichomes of even width. <i>Anabaena</i> type*	7
(c) Predominantly unicellular, but produce short trichomes and heterocysts when grown with a limiting supply of combined nitrogen. <i>Chlorogloea</i> type	1
(2) Do not produce heterocysts	
(a) Trichomes straight. <i>Oscillatoria</i> type†	6
(b) Trichomes helical. <i>Spirulina</i> type‡	2
(B) Unicellular	
(a) Cells spherical. <i>Gloeocapsa</i> type§	14
(b) Cells rod-shaped. <i>Synechococcus</i> type¶	12
* Includes strains possibly assignable to the form genera <i>Anabaena</i> , <i>Nostoc</i> and <i>Cylindrocapsa</i> .	
† Includes strains possibly assignable to the form genera <i>Oscillatoria</i> , <i>Phormidium</i> , <i>Lyngbya</i> , <i>Plectonema</i> and <i>Microcoleus</i> .	
‡ One strain not bacteria-free.	
§ Includes strains possibly assignable to the form genera <i>Gloeocapsa</i> , <i>Chroococcus</i> and <i>Aphanocapsa</i> .	
¶ Includes the strain frequently designated " <i>Anacystis nidulans</i> ".	

Cells were collected by centrifugation, washed and saponified in 50 per cent aqueous KOH-methyl alcohol-water (1:1:1) at 65°C for 1 h. After extraction of the non-saponifiable fraction with petroleum ether (boiling point 30°-60°C), the mixture was acidified and the fatty acids extracted with ethyl ether. Methyl esters were purified by elution from a silicic acid ('Unisil', activated silicic acid, 100-200 mesh, Clarkson Chemical Company) column with 5 per cent ether in pentane. Fatty acid methyl esters were analysed by vapour phase chromatography (Hewlett-Packard model 700 gas chromatograph) on 10 per cent diethyleneglycol succinate (DEGS) using helium as a carrier gas. Preliminary identification of fatty acids was by comparison of relative retention times with those of standards, and by the use of a plot of retention time against chain length¹⁰. The structure of the unsaturated fatty acids was confirmed by hydrogenation of the methyl esters¹¹ and oxidation of representative individual fatty acids purified by gas chromatography¹².

Table 2. DISTRIBUTION OF POLYUNSATURATED FATTY ACIDS AMONG BLUE-GREEN ALGAE

	Polyunsaturated fatty acid content: High	Low or absent	Ratio of saturated + monounsaturated fatty acids to polyunsaturated fatty acids
Filamentous forms:			
<i>Calothrix</i> type	1	0	1
<i>Anabaena</i> type	7	0	2
<i>Chlorogloea</i> type	1	0	3
<i>Oscillatoria</i> type	6	0	2
<i>Spirulina</i> type	0	2	> 59
Totals for filamentous types:	15	2	
Unicellular forms:			
<i>Gloeocapsa</i> type I	6	0	1
<i>Gloeocapsa</i> type II	0	8	> 21
<i>Synechococcus</i> type	0	12	> 13
Totals for unicellular types	6	20	

With respect to their content of polyunsaturated fatty acids, the strains fall into two readily distinguishable groups (Table 2). Some have a high content of these fatty acids, ranging from 27 to more than 50 per cent of the total fatty acids of the cell; others either do not contain these compounds, or contain very little of them (a maximum of 4 per cent of the total fatty acids of the cell). The strains which have a high content of polyunsaturated fatty acids have ratios of saturated + monounsaturated fatty acids to polyunsaturated fatty acids between 1 and 3, while the corresponding ratio for the remainder of the strains is 13 or more (Table 2). All filamentous strains examined belong to the high polyunsaturated fatty acid group, with the exception of two strains of *Spirulina*. It should be noted, however, that the presence of the γ -isomer of linolenic acid has been reported for the largest species of *Spirulina*, *S. platensis*⁸.

Polyunsaturated fatty acids are uniformly low or absent in rod-shaped unicellular blue-green algae of the

Synechococcus type. It should be noted that the unicellular strains previously found to lack polyunsaturated fatty acids belong to this structural type. The only typological group in our collection which proved to be heterogeneous with respect to its content of polyunsaturated fatty acid consisted of the coccoid unicellular blue-green algae (*Gloeocapsa* type): six of the strains examined had a high content of polyunsaturated fatty acids, while these compounds were undetectable or present in very small amounts in six other strains. As Table 3 shows, these two groups of strains differ markedly in DNA base composition, and are also for the most part readily distinguishable by cell size. Patterns of fatty acid composition for a few strains representative of the major types we have described are shown in Fig. 1.

Table 3. CHARACTERS CORRELATED WITH POLYUNSATURATED FATTY ACID CONTENT IN STRAINS OF THE *Gloeocapsa* TYPE

Type	No. of strains	Range of DNA base composition, moles per cent G+C (No. of strains analysed in parentheses)	Average cell diameter (μ m) range for all strains included
I	6	46.9-47.2 (6)	2.2-3.0
II	6	34.7-38.2 (4)	3.0-6.8

Another characteristic chemical property of the eukaryotic chloroplast is the presence of two types of glycolipids, mono and digalactosyl diglycerides². These compounds have not been identified in purple bacteria, which contain predominantly phospholipids. But a monogalactosyl diglyceride has been found in three species of green bacteria, and has been shown recently to be specifically localized in chlorobium vesicles, organelles of unique structure which contain the photopigment system of these bacteria^{5,13}. Earlier lipid analyses indicated that blue-green algae contain both mono and digalactosyl diglycerides^{7,14}. We made a cursory examination by thin-layer chromatography of the glycolipids in several representative strains, both filamentous and unicellular during this work. Every strain examined contained two glycolipids, with the staining properties and R_F values characteristic of mono and digalactosyl diglycerides.

Table 4. DISTRIBUTION OF LIPIDS AND FATTY ACIDS CHARACTERISTIC OF THE CHLOROPLAST AMONG PHOTOSYNTHETIC PROKARYOTES

Prokaryotic group	Mono-galactosyl diglyceride	Digalactosyl diglyceride	Poly-unsaturated fatty acids
Purple bacteria	—	—	—
Green bacteria	+	—	—
Blue-green algae, sub-group I*	+	+	—
Blue-green algae, sub-group II†	+	+	+

* Includes most unicellular representatives so far examined, together with a few filamentous forms.

† Includes the majority of filamentous representatives so far examined, together with a few unicellular forms.

With respect to polyunsaturated fatty acid and glycolipid content, the photosynthetic prokaryotes can accordingly be divided into four different groups (Table 4). The presence of a monogalactosyl diglyceride in green bacteria suggests glycolipid was introduced into the photosynthetic apparatus before the final step in the evolution of the mechanism of photosynthesis: the development of a type II photochemical reaction centre, operative in the oxidation of water. Among photosynthetic prokaryotes, digalactosyl diglycerides are confined to, but apparently universal among, forms that possess a type II reaction centre (that is, blue-green algae). The incorporation of this particular type of galactolipid into the photosynthetic apparatus may therefore have been associated with the evolution of type II reaction centres. The heterogeneity of blue-green algae with respect to the possession of 18:2 and 18:3 fatty acids suggests that these compounds became associated with the acyl lipids of the photosynthetic apparatus after the development of type II reaction centres, in some but not all lines of oxygen-evolving photosynthetic prokaryotes. This biochemical character was subsequently transferred, in association with the

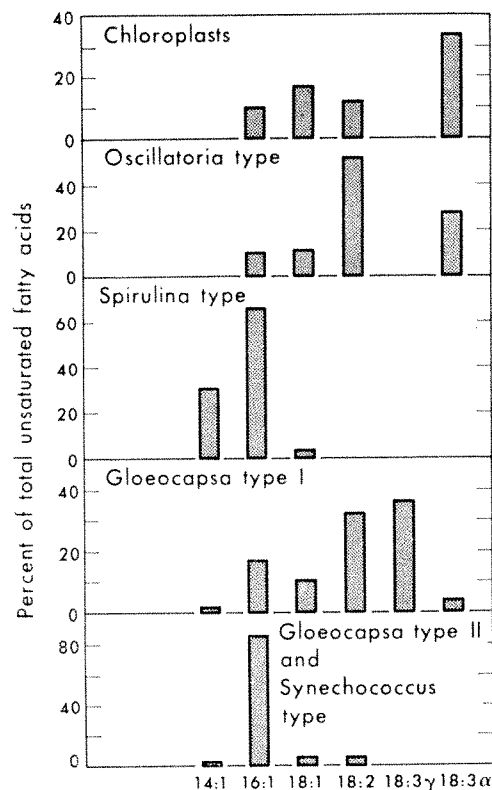


Fig. 1. The unsaturated fatty acids of chloroplasts and blue-green algae. Data are reported for chloroplasts of *Euglena gracilis* Z¹⁴ and for the following representative strains of blue-green algae: *Oscillatoria* type, strain 6506; *Spirulina* type, strain 6313; *Gloeocapsa* type I, strain W1; *Gloeocapsa* type II and *Synechococcus* type, represented by *Synechococcus* strain 6307. The fatty acids are described by carbon number (before the colon) and by the number of double bonds (after the colon). 18:3γ is octadeca-6,9,12-trienoic acid and 18:3α is octadeca-9,12,15-trienoic acid. The portion of the total unsaturated fatty acids represented as 18:3γ may be composed of a mixture of 18:3γ and another unsaturated 18-carbon fatty acid, possibly octadeca-12,15-dienoic acid.

machinery of oxygen-evolving photosynthesis, to eukaryotes.

The possible evolutionary affiliation between blue-green algae and eukaryotic algae (or their chloroplasts) is one of the most fascinating problems of cellular evolution. The unicellular blue-green algae are obviously a key group in this context. Our data on fatty acid content show that the organisms of the *Gloeocapsa* type with a DNA base composition of approximately 45 moles per cent G+C are the only unicellular blue-green algae known with a lipid composition like that of the chloroplast.

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Effect of Gross Pollution by Kerosine Hydrocarbons on the Microflora of a Moorland Soil

THE microbiology of the degradation of hydrocarbons in soil has been studied in some detail¹⁻⁴; as well as the many bacteria able to oxidize alkanes, certain fungi are thought to be involved to a significant degree in moorland soil⁵.

We have investigated the situation in two upland moorland sites called *A* and *B* in North Wales, which were polluted by the dumping of large quantities of an acidic sludge containing kerosine hydrocarbon. For analysis we used the method of Ramsdale and Wilkinson⁶. At site *A* the pH of contaminated soil was 2.0 and of uncontaminated soil was 4.6; at site *B* values were 1.0 and 4.2 respectively. The soil at site *A* was drier (moisture content, 71–175 per cent dry weight) than at site *B* (325–600 per cent dry weight). The sites were investigated during 7–10 months after the estimated date of pollution by methods based on those used previously^{6,7}. Samples were taken at the same level inside and outside the polluted zones; numbers of viable bacteria and fungi were estimated standard media for plate counting. Hydrocarbon oxidizing microorganisms were estimated by membrane filtration of a suitably diluted soil sample followed by incubation of the filter at 10° C on a sterile glass fibre filter pad, soaked in a mineral salts medium to which either *n*-dodecane or crude oil diluted in *n*-hexane had been added. Plates were counted after 8, 14 and 16 days. The samples were analysed in a Gilson respirometer at 10° C. The results are the means of three samples at each site.

The microflora of the two sites responded differently to pollution by the kerosine. At site *A* bacterial and fungal counts declined significantly ($P=0.05$), whereas at site *B* there was no significant difference in bacterial counts, and fungal counts increased ($P=0.05$) (Table 1). Estimates of hydrocarbon oxidizers (Table 2) showed the same trend with a more marked response to hydrocarbon pollution at site *B*. Fungi able to metabolize hydrocarbons increased most here ($P=0.01$) regardless of the hydrocarbon test substrate used. Oxygen consumption was not affected at site *A* (Table 3) but was stimulated to some extent by pollution at site *B*. The possibility of anomalous results due to a requirement for CO₂ by certain microorganisms was overcome by determination of total gas exchange. The calculated CO₂ evolution confirmed the stimulation of respiration in the polluted zone of site *B*. The addition of an emulsion of *n*-dodecane in distilled water to unpolluted soils inhibited respiration at site *A* but stimulated it at site *B*, indicating the ability of a proportion of the latter population to metabolize hydrocarbons. With soils from polluted zones results were variable and contradictory, and we are developing more reliable methods for estimating rates of hydrocarbon metabolism.

Estimates of soil respiration due to hydrocarbon metabolism have been extremely variable². Some of the difficulties in interpretation may be a consequence of

Table 1. ESTIMATES OF VIABLE BACTERIA AND FUNGI AT SAMPLING SITES

Sample	Fungal counts x/g dry wt	Bacterial counts x/g dry wt
Site <i>A</i> clean	41 x 10 ⁴	640 x 10 ⁴
Site <i>A</i> polluted	11 x 10 ⁴	34 x 10 ⁴
Site <i>B</i> clean	76 x 10 ⁴	640 x 10 ⁴
Site <i>B</i> polluted	420 x 10 ⁴	590 x 10 ⁴

Table 2. ESTIMATES OF HYDROCARBON OXIDIZING BACTERIA AND FUNGI

Sample	<i>n</i> -Dodecane	Crude oil	<i>n</i> -Dodecane	Crude oil
Site <i>A</i> clean	54 x 10 ³	56 x 10 ³	15.2 x 10 ³	25.4 x 10 ³
Site <i>A</i> polluted	8.2 x 10 ³	10 ³	5.5 x 10 ³	5.5 x 10 ³
Site <i>B</i> clean	46 x 10 ³	21 x 10 ³	8.4 x 10 ³	10 ³
Site <i>B</i> polluted	385 x 10 ³	252 x 10 ³	14 x 10 ³	7 x 10 ³

Table 3. RATES OF RESPIRATION OF SOIL SAMPLES

Sample	Mean rates (μl. of gas/h/g dry wt)	Percentage inhibition (–) or stimulation (+) in respiration on addition of <i>n</i> -dodecane
Site <i>A</i> clean	13–22	–12 to –60
Site <i>A</i> polluted	12–30	–5 to +5
Site <i>B</i> clean	20–46	+7 to +20
Site <i>B</i> polluted	50–80	–1 to –8

a limitation on mineral nutrients which resulted from removal to the laboratory, for upland peats such as these derive much of their mineral nutrients from rainfall. There is indirect evidence for this in the lack of response of microflora at the drier site and the pronounced adaptation in numbers of hydrocarbon oxidizers at site *B*, which is well flushed with water and presumably with mineral nutrients. The low pH of the oil spillage has presumably played some part in the response of the fungi.

The frequency of spillages on land in recent years led us to try a simple qualitative test for the response of fungi in soils. Small soil samples (about 5–10 g wet weight) were placed in sterile disposable Petri dishes and 0.5 ml. of test hydrocarbon was pipetted on to the soil, and the dishes were sealed. After various test conditions had been tried, we incubated this soil for 10 days at 20° C with *n*-dodecane as substrate. No fungal hyphae grew on site *A* soils (polluted or clean) but there was heavy growth on the clean soil of site *B*, indicating the ability of the fungal population at this site to adapt to hydrocarbon oxidation, and the polluted soil of site *B*, indicating the absence of inhibition by the spillage. There was no growth on control soil samples to which hydrocarbon had not been added.

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Book Reviews

PHYSIOLOGIST'S PHILOSOPHY

Mind and Brain

A Philosophy of Science. By Arturo Rosenblueth. Pp. xii+128. (MIT: London and Cambridge, Massachusetts, April 1970.) 56s.

ROSENBLUETH'S principal topic in this book is the mind's relation to the physical world, although he examines a number of other problems of the philosophy of science. There is a good discussion of the nature of experiment in the light of Claude Bernard's *Introduction à l'Étude de la Médecine Expérimentale*, and a much less good examination of what types of inference are important in science. Rosenblueth has been most influenced by the works of Eddington, Russell, and Schrödinger. He is himself a neurophysiologist; he studied under W. B. Cannon and was a colleague of Norbert Wiener with whom he ran a seminar on scientific method at Harvard in 1930. The cyberneticist's approach to the concepts of psychology is not, however, in evidence here.

Rosenblueth presents a comprehensive summary of those parts of biology and physiology which are relevant to mental phenomena in order to establish two theses. First, that no mental properties enter into the laws of science: "the only determinism that I recognize is that of physics". Second, "that mental events are invariably correlated with physiological processes in the brains of the animal organisms that experience them". He does not regard this correlation as a chance one: "Our sensations are causally related to events that occur in a material universe of which our own bodies are a part". The first thesis is supported by a discussion of genetics which leads to the rejection of Schrödinger's vitalist view that the physics of the matter of living organisms will turn out to differ from that of other matter, and by a discussion, and rejection, of the possibility that the human will controls the body by influencing physical events which other physical events leave indeterminate. The second thesis is supported by a discussion of the physiology of the central nervous system and of the sense organs. Rosenblueth accepts Russell's view that our knowledge of material events is confined to their structure. He concludes that this knowledge depends on the production in the cerebral cortex of a system of events isomorphic in structure to the events of the physical world external to our bodies. It is these cortical events which are directly correlated to mental events.

The great shortcoming of the book is that no adequate review of the facts and concepts of psychology is set alongside that of the facts and concepts of biology. There is, however, a short discussion in which Rosenblueth argues for his third principal thesis: that mental events are totally distinct from any physical events. In particular he argues for a fundamental difference between mental events and their correlated events in the nervous system.

Rosenblueth argues persuasively for each of these three theses, but they are mutually inconsistent. If mental events are fundamentally distinct from the physical and yet they are interdependent, then the interdependence

must be a causal relation between mental and physical, so not all determination will be physical. He recognizes this inconsistency. He seems to suppose that it can be partly resolved by regarding mental and neurological events as "correlates" of one another, rather than as causes, and, again, on the last page but one of the book, he states that his views lead to the conclusion that "a mental process and the neurophysiological phenomena that underlie it represent two different aspects of a single event". No mention is made of the considerable discussion which the thesis that mental events are identical with brain processes has received in the past ten years from such writers as U. T. Place, J. J. C. Smart and D. M. Armstrong. In the absence of further discussion, these phrases—"correlates" and "aspects of a single event"—recognize an interdependence between the mental and the physical which is not that of causality, nor that of logic, but go no way to explain it.

JOHN WATLING

MEASURING THE BOX

Television

The Ephemeral Art. By T. C. Worsley. Pp. 255. (Alan Ross: London, 1970.) 50s.

Media Sociology

By Jeremy Tunstall. Pp. ix+574. (Constable: London, 1970.) 30s.

The Effects of Television

Edited by James D. Halloran. Pp. 224. (Panther: London, 1970.) 10s.

THE effects of television have been exaggerated. But they are none the less real for being elusive. These three interesting books, while destroying the notion that programme makers and advertisers can manipulate the minds of viewers, present a theory that is just as uncomfortable. It is that what people learn from their television sets is not at all what they have been thought to have been learning. If they have been misled, it is down different paths than the television-haters feared. The poor, for example, seem not to look to television for escape from their drab lives, but for a picture of how the other half lives—not for fantasy but for information. The same obliqueness applies to television violence. It is not how savagely the chair breaks over the cowboy's head that impresses the impressionable viewer; it is who throws it—good guy or bad guy—that counts.

Mr James Halloran, director of the Centre for Mass Communication Research at the University of Leicester, has contributed a comprehensive essay on research into the social effects of television as a lead piece to a collection of five on the subject. He argues that the temptation to over-simplify must be fought at every turn. It is too easy to say that lonely children rely on the television set for friendship, or that television can be studied as an isolated phenomenon that may or may not encourage delinquency. Television has to be placed in context with other forms of mass communication and other social patterns that may lead to deviant behaviour. There is an impressive amount of research, both in Britain and in the United States, now trying to do just this, and Mr Halloran surveys it nicely.

One of the most identifiable effects of television may have been to keep alive the British Liberal Party. Dr Jay Blumler of the University of Leeds shows, in the same volume, that the influence of television is greatest on those who are least interested. The swing of opinion in 1964 in favour of the Liberals seems to have been related to the exposure of that party on television to uncommitted viewers who could not bother to turn off their set. For better or worse, television seems to be most effective in dealing with topics remote from the viewer's own

experience. (Perhaps India is right in worrying about the impact in Britain of the Louis Malle series.) Television also has another fairly unrecognized power—that of reaching beyond leaders to the rank-and-file. In recent years both trade unionists and Roman Catholics have come to differ sharply with their leaders' views; Dr Blumler thinks television may be in part responsible.

Any serious student of sociology or mass communication will find Jeremy Tunstall's *Media Sociology* necessary for reading and reference. Others will find it heavy going. Featureless, voluminous, ill-assorted, it embraces everything from the sociology of bookshops to Soviet television news, yet it could well be a gold-mine for those struggling to keep up in a maddeningly amorphous field.

One humanist may succeed where a dozen sociologists fail. Mr T. C. Worsley, the television critic of the *Financial Times*, has produced a collection of his reviews over the past few years which tell more about the effect, intended and unintended, of British television than does a mountain of sociological prose. I praise him somewhat guiltily. My children accused me of hating Alf Garnett until I read in the *Financial Times* that he was Falstaffian. Yet on re-reading the original Worsley appraisal of "Till Death Us Do Part", how right I was to be persuaded. Alf Garnett, judged Mr Worsley,

"is everything most hateful about our national character—xenophobic, illiberal, racist, anti-semitic, toadying, authoritarian. . . .

"How is it then, you may ask, if he is so despicable, that Mr Speight manages to make of him something so appealing as well as so funny? But isn't just this the peculiar virtue of the comic genius that it at the same time exposes vices to our laughter and allows us to love the creatures who embody those vices? Maddening though it must be for Mrs Whitehouse, the more cowardly, bragging and dishonourable Falstaff is, the more we love him. It is the same with Alf Garnett".

Mr Worsley casts the same intelligent, discerning, unsnobbish eye on a number of memorable television programmes; he sees them in terms of challenges to the medium as well as to the authors and performers. "Z-Cars" brought a flash of creative observation to a familiar set-up; Shakespeare is the most difficult of authors to get across on television, for his language is, initially, a device to bridge the gap between stage and audience and it is just this gap which television bridges anyhow. Jonathan Miller's "Monitor" never recovered from the impact of Miss Susan Sontag, the walking parody of the contemporary intellectual (American style).

BRENDA MADDOX

SOCIAL GROUPINGS

Social Behaviour in Birds and Mammals

Essays on the Social Ethology of Animals and Man. Edited by John H. Crook. Pp. xl+492. (Academic: London and New York, June 1970.) 140s.

Most students of behaviour aim to relate their subject matter to events which precede it in time, and which may lie at a finer (physiological) level of analysis—in brief, to its causes. Recently the importance of relating behaviour to its consequences, particularly its adaptive consequences, has been emphasized by Tinbergen and others. This book, by a group of postgraduate workers from Bristol, represents yet another direction of gaze—towards more complex levels of organization. It is concerned in particular with the social bases and social consequences of individual behaviour and the behaviour of social groups in relation to their environment. In his editorial introduction Dr Crook argues that ethologists' concern over inter-individual relations and lower verte-

brates can lead to an emphasis on social structure and behaviour as fixed characters, changeable only through natural selection, which is at variance with the role of tradition found in recent studies of sub-human primates. This of course has considerable bearing on the naive extrapolations from animal to human behaviour by "some recent authors". Furthermore, Crook emphasizes the need to formulate the precise relationship between studies of individuals and studies of social organization, and to delineate the ways in which they can enlighten each other.

The first four essays are concerned with the relations between ecology and social organization in birds and primates. The two bird studies differ in their approach. Goss-Custard assesses the determinants of the dispersion of flocks of wading birds, using quantitative data to relate dispersion to behaviour and feeding efficiency. Simmons describes the behaviour of two fish eating birds, the brown booby and (in less detail) the great crested grebe, showing how their breeding biology and social organization are related to ecology. The latter follows the traditions set by Tinbergen and his colleagues for gulls, and Crook's own earlier work, based on extensive data on many weaver-bird species. It is unfortunate that such data are not available for sub-human primates. Moreover, as Aldrich-Blake's admirable chapter shows, much of the published information on primates is based on quite inadequate field methods. For this reason Crook's stimulating review of the socioecology of primates is valuable more as providing a programme for future research in this field than for the generalizations about relations between behaviour and ecology which it contains. Crook also discusses the role of the social environment in behavioural ontogeny, and the factors influencing stability and change in social structure, providing thereby a valuable review of many recent field studies.

The second section is concerned with certain aspects of social behaviour. Archer reviews data on the effects of population density on behaviour in rodents—sexual, maternal and exploratory as well as the more usually studied agonistic behaviour and physiology. Crook and Butterfield discuss the endocrine basis of aggressive behaviour in a weaver-bird. They then show how sex differences in aggressiveness may affect the sex ratio, and how this is related to the social structure and to the relatively severe "over-wintering" environment. Butterfield also presents a study of the pair-bond in zebra finches, relating its permanence to the species ecology and examining its basis in a series of laboratory studies. Finally, Vine reviews facial-visual communication in both human and sub-human species, demonstrating the complexity which underlies even something so apparently simple as "gazing".

The third section is concerned with problems of development. Kear provides an important review of parent-young interaction in waterfowl, relating its evolutionary radiation to other characters of behaviour, and thereby providing a link with the themes in section one. Cronhelm reports some experiments on stimuli eliciting pecking in young chicks, finding complex interaction effects between stimulus characteristics as more variables are introduced. She also demonstrates the potential importance of observational learning in affecting pecking rate in an operant situation. Finally, Dimond describes experiments showing that exposing hens' eggs to light before hatching affects diverse aspects of the chicks' post-hatching behaviour, queries current theories of imprinting and offers some speculations on the organization of perceptual-motor functions and the development of fear.

The essays thus range from reports of previously unpublished research to wide-ranging reviews. Though they are heterogeneous, each one is of merit, and Crook's introduction goes a considerable way towards welding them into a coherent whole.

R. A. HINDE

LIFE BETWEEN THE TIDES

Biology of Intertidal Animals

By R. C. Newell. Pp. viii+555. (Logos in association with Elek Books: London, 1970.) 160s.

THIS is a very welcome book on the biology of intertidal animals and will undoubtedly prove most useful to students of zoology and marine biology. Obviously designed for this purpose, it is regrettable that the publishers have produced it at a price which few students can afford. A paperback edition for £3 or less would surely have been possible, and it is hoped that the publishers will think again.

This "Biology of Intertidal Animals" is an individual account with special emphasis on aspects which clearly interest the author most: in this it is stimulating, but it is perhaps not as comprehensive as its title, bulk and price might suggest. After an introduction on the physical, chemical and biological features of the intertidal zone, there follows a chapter on the faunas of special habitats—crevices, seaweeds and rockpools—which will be particularly useful to students on field courses. Chapter 3, on the establishment of zonation patterns, is a useful summary of recent research on larval settlement, and the chapter which follows on the maintenance of these patterns of zonation presents an interesting summary of the behavioural aspects of such common animals as the species of *Littorina*, *Lasaea*, *Talitrus* and *Hydrobia*. Mechanisms of feeding, factors affecting feeding; respiratory mechanisms; factors affecting the rate of oxygen consumption; thermal stress and desiccation, form subjects of other chapters. Inevitably, one must compare this book with J. A. C. Nicols's earlier and successful *Biology of Marine Animals* now available as a paperback. Nicols's book is not restricted to intertidal animals and is therefore wider in one sense in dealing more with fishes and physiology, and on the other is more limited in the detail on intertidal forms. Newell's book is therefore welcome because its approach is very different, especially in descriptions of behaviour: Nicols's approach is essentially that of a comparative physiologist; Newell is evidently more interested in the animal in the field.

One of the chief difficulties facing an author of a book of this kind is how far to describe the basic information on which the real discussions are based and how much to assume what is known by the reader or is available to him elsewhere. It is a matter of opinion whether "the process of respiration" (pp. 267–283) or the account of the general properties of respiratory pigments (p. 302) are really necessary, or whether this information should more properly be sought in a textbook of physiology in order to follow the discussions on "oxygen stores" and "anaerobiosis". Similarly, the account of learning in nereids (p. 155) seems to me to be a digression in an otherwise valuable and coherent section. One might also ask if chapter 5 describing feeding mechanisms is really necessary in order to provide the background necessary to the chapter which follows on "Factors affecting Feeding".

To me the most interesting sections were those on maintenance of zonation patterns, thermal stress and desiccation, and the factors affecting feeding and respiration, and I would rather have seen the approach adopted in these chapters extended to some other topics which have been omitted or dealt with rather briefly than to have these other sections describing the basic facts. There is nothing on nitrogenous excretion and little on osmotic stress or the factors of importance in intertidal estuarine habitats. There is no mention of the uricotelic metabolism of *Littorina neritoides*, for example, nor is there any account of Trueman's work on burrowing in certain bivalves or in the lugworm, or of the microfauna, of population ecology or of the adaptation and control of the reproductive method. In spite of these omissions, as the author says in his own preface, this book is "an

attempt to account for the distribution of intertidal animals in physiological terms" and it will undoubtedly find a place in all departments of zoology where animals are still studied. It is a well produced book with clear illustrations and tables placed appropriately in the text. This is a book to stimulate students to further study of the biology of intertidal animals and to enlarge the horizons of those attending marine courses who may not have had the opportunity to keep abreast of the literature which the author so ably reviews in the light of his own work.

R. PHILLIPS DALES

FISH PARASITOLOGY

Aspects of Fish Parasitology

Edited by Angela E. R. Taylor and R. Muller. (Symposia of the British Society for Parasitology, Vol. 8.) Pp. vii+167. (Blackwell (Scientific): Oxford and Edinburgh, 1970.) 60s.

THE parasitology of fishes as an area of research has been enjoying a rapid and very gratifying recent expansion. Some investigations have concentrated on commercial species and on parasites obviously harmful to the host. Others—particularly those concerned with helminths and copepods—have been taxonomically oriented, and often have ignored the fish host. Still other studies, notably those in the Soviet Union, have displayed strong ecological emphasis. Notwithstanding, major gaps remain in our knowledge of the parasites of fishes. In the marine environment particularly, many species are as yet undescribed, not to mention the many host species whose parasite fauna has not received even cursory attention. New impetus for studies of parasites and diseases has been generated because of the expansion of aquaculture of fishes, and the predictable concurrent expansion of parasite and disease problems in species under cultivation. Other research areas deserving of more attention include the life cycles and ecological relations of helminths, helminth physiology, and the impact of parasites on host mortality rates.

This volume is a valuable addition to the burgeoning literature in fish parasitology. It contains a collection of six thorough and lucid papers—helminthological in emphasis—on parasites of certain freshwater and marine fishes. Papers by K. MacKenzie and D. Gibson and by H. H. Williams *et al.* provide valuable insights about the relation of the morphology, physiology and chemistry of the host gut to the helminth fauna of microenvironments within that habitat. MacKenzie and Gibson have also summarized available information, including new experimental data, about the life histories of several common helminth parasites of plaice and flounders. The physiology of fish parasites, last summarized in 1961 by Markov, is reviewed succinctly by C. Arme and M. Walkey. Emphasis is placed on new information derived during the past decade from *in vitro* and transplantation studies. A few of the more interesting crustacean and molluscan parasites of freshwater fishes have been considered by G. Fryer as examples of how such organisms contribute to the solution of zoogeographical problems. J. C. Chubb has sketched broadly both the present status of knowledge and the need for additional information about the parasites of British freshwater fishes. He has examined available parasitological data from oligotrophic, mesotrophic, and eutrophic lakes, and from various zones of flowing waters, comparing the parasite faunas of salmonoid and cyprinid fishes. C. R. Kennedy has described a very interesting systems approach to the population biology of helminths in freshwater fishes which is illustrated by flow diagrams of representative host-parasite systems.

Although of primary interest to parasitologists and fishery biologists, the articles should also benefit specialists

in other biological sub-disciplines. The physiologist may find unexplored vistas of research in fish parasites; the zoogeographer can find examples of an additional, possibly powerful, tool in the form of fish parasites; and the population biologist may see an added dimension to his studies of free-living animals.

CARL J. SINDERMAN

INTRODUCTION TO PLANT GROWTH

The Control of Growth and Differentiation in Plants
By P. F. Wareing and I. D. J. Phillips. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Botany Division.) Pp. x+303. (Pergamon: Oxford and New York, May 1970.) 50s (\$7) board; 40s (\$5.50) flexi-cover.

THIS widely ranging book is chiefly intended as an introduction to plant growth and development for undergraduate students. As its title suggests, its emphasis is on control, and particular attention is given to the role of natural regulators of growth and differentiation processes. The first three chapters describe the characteristics of cell growth and differentiation, and show how they are related to the morphogenesis of tissue and organisms. Then follow four chapters on the nature of plant growth hormones and their respective roles in the growth of stem and root, including secondary growth, in bud and fruit growth and in abscission. Hormones and growth movements occupy one chapter and a short chapter is included on the sterile culture of cells and organs. Two long chapters outline the complexities of the physiology of flowering and there are two excellent chapters on dormancy and senescence respectively. A final chapter summarizes in simple terms current modern ideas on the genetic aspects of development control. Each chapter ends with a list of about a dozen review articles for further reading.

This book should serve as an excellent textbook for undergraduates taking basic courses in plant physiology. More advanced undergraduates specializing in this area will find it a useful guide to further review articles in this field. They may, however, also find rather distressing, as I did, the deliberate omission of supporting evidence for all but a few statements, and a complete lack of reference to individual pieces of research work. Admittedly the over-larding of the text with authors' names and dates of publication does tend to interrupt the logical flow of an argument, but an important function of a textbook is to stimulate the student to read the important key research papers—to see for himself how fundamental advances are made. This book should stimulate, but it will not help him to do this. The book is clearly but not overlavishly illustrated with carefully chosen diagrams and photographs. It has a pleasant format and suffers from very few errors (for example, the misnaming of *Lunaria* on pages 201 and 204).

L. J. AUDUS

DISEASES OF ROOT SYSTEMS

Pathogenic Root-infecting Fungi

By S. D. Garrett. Pp. xi+294. (Cambridge University: London, July 1970.) 75s; \$12.50.

THIS book is a sequel to Dr Garrett's *Biology of Root-infecting Fungi* published in 1956. The very high standard to which we are accustomed from all his previous publications is maintained. This work will be invaluable not only to the specialist but also to students of botany.

Although the author states that the book can be read through, and this is very true, it can certainly be used as a reference work because the bibliography is extensive and the subject matter is very comprehensive. The style of presentation is lucid and authoritative.

The author is not merely an academic mycologist/plant pathologist; he makes it clear that his studies are economically important as well as fundamental. Throughout the book he constantly brings home the important truth that "diseases of the root systems cannot be studied in isolation as a simple interaction between root system and fungal pathogen", and he stresses that an appreciation of what is going on in the soil is fundamental to an understanding of the disease.

In an excellent introductory chapter, problems are surveyed and definitions of terms are proposed. The discussion on inoculum-potential and the concept of "energy of growth" available in the fungus to cause infection is particularly interesting. Unspecialized parasites are discussed in the second chapter and this is followed by two chapters on the specialized parasites—one dealing with vascular wilt fungi and the other with ectotrophic root-infecting fungi. The saprophytic activities of these organisms are then considered under the headings of competitive saprophytic colonization of substrates and saprophytic survival in infected host tissues. Then follows an excellent survey of dormant survival by resting propagules, spores and sclerotia of these fungi. The last chapter deals very thoroughly with principles of control of root diseases of field crops, intensive crops and plantation crops.

There are twenty-five diagrams present as contrasted with their virtual absence in *Biology of Root-infecting Fungi*. In this earlier book, Garrett stated that what is evident in the study of plant pathology "is a paradox of biology today; it is divided up as never before, yet never before, perhaps, has its unity seemed more apparent to us". In his new book he goes a long way to make the unity of plant pathology real; he has once again produced what may be termed a philosophy of plant pathology.

Pathogenic Root-infecting Fungi is a mine of information and has a very useful index. The production is excellent. It is most strongly recommended.

IVOR ISAAC

PHOTOSYNTHETIC RESEARCH, 1968

Progress in Photosynthesis Research

Edited by Helmut Metzner. Vol. 1: Structure of the Photosynthetic Apparatus, Physiology of Photosynthesis. Pp. xv+1-536+37. Vol. 2: Plastid Pigments, Electron Transfer. Pp. xi+537-1128. Vol. 3: Photophosphorylation, CO₂ Fixation, Action Mechanisms of Herbicides. Pp. 1129-1887+37. (Verlag C. Lichtenstein: Munich, 1969.) 359s 6d the set.

THESE volumes contain the papers read at an international conference on photosynthesis. The book, like the conference, covers a wider range of photosynthetic research than any other recent symposium which deals with the topics at comparable depth. There are, however, notable gaps in its coverage of modern research. It would have been useful to have a paper on experiments with heavy oxygen in the section on photorespiration, along with the papers on tracer carbon and net gas exchange techniques. Another lacuna was the absence of a paper on the biochemistry of plants which fix CO₂ by way of the C₄ dicarboxylic acid pathway. Finally, the central dogma of photosynthesis, the "Z scheme" for non-cyclic photophosphorylation, was only attacked by Arnon. More severe criticisms of its basic tenets, such as Arnold with his solid state model, and the Warburg-Vennesland-Stiller view that interaction of CO₂ with chlorophyll is the primary event, were not represented. I do not wish to

criticize the organization of the symposium, but only to comment that the absence of these viewpoints necessarily makes the volumes less useful as a summary of current thinking on photosynthesis.

In any case, is it worthwhile to review current thinking on photosynthesis in book form, rather than to rely on publication in journals? This is especially important in view of the interval of 18 months between the conference and the appearance of the book in Britain. This lag means that the book is inevitably dated by the time it appears; as an example, Arnon's heretical views have subsequently undergone schism¹.

By putting a wide range of photosynthetic research into these three volumes, it is possible that some cross-fertilization will occur between different approaches to photosynthesis. It may be argued that the profitable cross-fertilization occurs laterally, with, for example, physical chemistry, mitochondriology and micrometeorology, rather than between, say, the physico-chemical and ecological approaches to photosynthesis itself. Still, it is as well to leave the doors open. The specialist in one aspect of photosynthesis can find, in these volumes, a summary of the conclusions reached by contributors to other specialized sections, and provides entry to current research in other fields. These volumes thus develop their theme at two levels of complexity, and are the more useful for that.

Cross-fertilization can be aided by the excellent index, repeated in each volume, which makes the following of one particular point (for example, the mode of action of an inhibitor) easier than would the presentation of the same papers in a variety of journals, abstracting journals notwithstanding. One important feature often found in symposium reports, but lacking in this instance, is a record of the verbal discussion which followed each paper. These discussions often place the work in a wider context, as well as pointing out directly relevant technical or conceptual points. One can but hope that at least some of the points raised in discussion have been incorporated into the papers as printed in these volumes.

In those sections in which I have greatest knowledge of the literature, it would seem that about half of the papers in these volumes are substantially different from papers which have appeared in journals. The overlap, and the importance of the time lag in publication, is greater for papers on electron transport and energy coupling than for, for example, photorespiration and photophosphorylation *in vivo*. To this extent the volumes are more useful as a unique source in these less central topics in photosynthetic research. Unfortunately, there is no way of determining how much of the interesting material exclusively found here would have appeared elsewhere if these volumes had not been published.

Assuming that this material would have been published elsewhere, are these books more useful than journal publications, together with some literature survey (such as the one in *Photochemistry and Photobiology*, which has about the same total time lag, that is publication of paper and appearance in the literature survey, as does the appearance of the papers in this book)? It is convenient to have the papers collected together in bound form, together with the index and interpretative summaries, although more wide-ranging summaries occur in other review articles. It is useful as a source for research workers, university teachers and (possibly) advanced undergraduates. Few will be able to afford it, unless they took advantage of the pre-publication subscription offer; it must undoubtedly be available in university and research institute libraries.

Let it not be thought that I am damning with faint praise; within the limitations of the bound book format, Dr Metzner has done very well in assembling the very large number of papers into three such attractive and well produced volumes.

JOHN RAVEN

Knaff, D. B., and Arnon, D. L., *Proc. US Nat. Acad. Sci.*, **64**, 715 (1969).

RADIOLOGICAL RECIPES

Manual on Radiation Dosimetry

Edited by Niels W. Holm and Roger J. Berry. Pp. xvi + 450. (Dekker: New York, April 1970.) \$24.50; 233s.

RADIATION dosimetry is an art which, like cookery, depends on well tried and tested recipes carefully prepared and elegantly presented. This rather expensive manual provides twenty step-by-step recipes supported by reviews of the principles on which they are based. But who will use it? The editors give a clue by selecting "areas" of application—radiation protection in hospitals, radiation therapy and the industrial use of cobalt-60 sources and accelerators.

This manual lacks adequate cross-referencing and the editors do not impose consistent terminology on the authors of individual chapters. Their own introduction omits the recommendations of the International Commission on Radiological Units and Measurements¹ which must surely be taken as the authority on terminology. Readers would do well to turn to W. L. McLaughlin's chapter on "Films, Dyes and Photographic Systems" in which they will find a well referenced survey of the "jungle" of hundreds of available dosimetry systems.

T. E. Burlin's treatment of dosimeter response has a few small and obvious errors, but this is a clear and easily read chapter which could be usefully expanded to cover detectors other than ionization chambers. This chapter and that on calorimetry are not supported by recipes, although these fundamental dosimetry methods require at least as much skill as the more obvious cookery book methods. Abundant recipes and advice are offered on aqueous dosimeters, leaving little comfort for those readers interested in non-aqueous systems (such as nitrous oxide), which may be better for the measurement of high total dose.

The editors were fortunate to have contributions from J. R. Cameron and F. M. Attix on "Radiophotoluminescent and Thermoluminescent Dosimetry"—a brief reference to thermally stimulated electron emission would have completed a thorough survey of this important dosimetry field.

The use of anatomical phantoms in dosimetry deserves more than the brief mention by L. H. Lanzl, who describes the application of dosimetry to medical radiation therapy. Future editions would benefit from an examination of the tissue equivalence of phantom material and available phantom systems.

The recipes in this manual, taken with the principles, are suitable for users of gamma sources, particularly cobalt-60 and accelerators. It is of lesser value to reactor health physicists who will find little reference to neutrons and none to mixed field dosimetry. Future editions will be improved by more severe editing, and a sound system of terminology would raise this book of favourite recipes to the Escoffier class.

J. R. A. LAKEY

¹ *Radiation Quantities and Units*, Report 10a of the International Commission on Radiological Units and Measurements. (NBS Handbook 84.) (US National Bureau of Standards, Washington DC, 1962.)

PRINCIPLES OF BAND THEORY

Band Theory of Metals

The Elements. By Simon L. Altmann. Pp. xiv + 250. (Pergamon: Oxford and New York, June 1970.) 50s (\$6.75) board; 35s (\$4.75) flexi-cover.

THE theory of the solid state is dominated by Bloch's theorem: an electron in a regular crystal lattice must have wave-like quantum states, each of a specific wave vector. The proof of this theorem, and an examination of its consequences, first in one-dimension, then in more complicated three-dimensional lattices, is indispensable to any understanding of the modern theory of metals and semiconductors. The mathematics needed is not very

sophisticated—solid geometry, vector algebra and Fourier analysis—but the subject has a language of its own which must be acquired by deliberate study. Nobody being drawn into the great maw of “materials science” can afford to be ignorant of the conceptual apparatus of Brillouin zones, Fermi surfaces, and the like.

Dr Altmann has attempted the most difficult task of a teacher: he has written a book that is both simple and precise. He assumes no more than the elementary mathematics I have mentioned, and yet gives a careful and complete account of the basic principles of this theory. One can judge the didactic power of such a book only by practising with it on defenceless students; but it seems very good indeed. The emphasis on the simple symmetry properties of wave functions is especially helpful, and would be an admirable introduction to more advanced group theoretical methods in this field.

This is, of course, essentially a textbook (third year undergraduate or beyond), so that there is little new to say. I doubt the value of a whole chapter on quantum mechanics, which can scarcely be considered a mere auxiliary to the physics of metals, but it serves to define the essential terms and principles. I am also completely opposed to the retention of 2π in wave-like exponentials, but Altmann gives his reasons and this is the sort of arbitrary quarrel that can only be settled by a toss of a coin—or by cold steel! The book is perhaps weakest in the final chapters where the connexions of the mathematical theory with experimental physics are demonstrated by a few examples. Alas, the classical explanation of the Hume-Rothery rules looks very thin nowadays, as the author admits, and electronic specific heats are not very convincing as mere numbers. The trouble is that the theory really comes into its own in the study of Fermi surfaces, which demands, of course, a whole lot more physics than can be accommodated here, while the actual calculation of band structures is a complete art on its own. But the book can be most warmly recommended for the excellent exposition of the principal theme.

J. M. ZIMAN

ATMOSPHERIC TIDES AND WAVES

Atmospheric Tides

Thermal and Gravitational. By Sidney Chapman and Richard S. Lindzen. Pp. ix+200. (Reidel: Dordrecht, 1970.) 38 florins.

THERE cannot be many examples in the geophysical sciences where such a minute effect as the 12-hourly barometric pressure oscillation at sea level has led to an almost continuous discussion in the literature during the best part of two centuries. In their book, Chapman and Lindzen give an excellent account of the various efforts to explain the predominance of the solar semidiurnal over the corresponding lunar oscillation, because the latter should have the largest amplitude if the effect is understood as the result of air motions similar to the gravitational tides of the sea. Already, at an early stage, thermal excitation had been considered but, because of the large diurnal term in the heating function, a 12-hour resonant mode was ascribed to the atmosphere in order to account for the observational picture. The book describes how, on the one hand, the theory of atmospheric tides became more and more sophisticated and how, on the other, observational data made its validity more and more doubtful.

About 20 years ago the theory seemed perfected, only to be found untenable shortly after in the light of newly found atmospheric parameters. The experimenter in the field was no little alarmed by this state of uncertainty and the situation has only just become more relaxed as a result of the sensible use of rocket measurements in conjunction with the dynamic theory of atmospheric tides. At the present stage the critical reader of this book,

having studied the various sections on dynamic theory and having acquainted himself with the observational data, should no longer be surprised by the amplitude of the solar semidiurnal mode in the daily pressure pattern.

Although much attention is given to atmospheric phenomena at ground level, the book is also generally concerned with travelling waves and diurnal oscillations of solar as well as lunar excitation in the whole range of atmosphere up to ionospheric heights. The sections on methods of data analysis are of special interest to the experimenter who will also benefit from the treatment of mathematical methods dealing with various theoretical equations relevant to his work. As far as the presentation of observational material is concerned, the book is somewhat heavily biased towards meteorological data applying to the lower atmosphere, and it is regrettable that comparatively little space is devoted to upper atmospheric parameters, particularly those obtained with the various radio techniques.

Considering that the last monograph on the subject dates back more than 20 years the book is certainly meeting a long-standing demand for a comprehensive summary of present-day understanding of tidal phenomena in the terrestrial atmosphere. It is clear that the authors prepared chapters individually, including numerous references to their own specific investigations. Within the available space they have managed to concentrate on the principal features of the subject without neglecting important detail, and there is an exhaustive list of references for those who wish to go into more extensive studies.

H. G. MULLER

EMPIRICAL BAYES

Empirical Bayes Methods

By J. S. Maritz. (Methuen's Monographs on Applied Probability and Statistics.) Pp. viii+159. (Methuen: London, May 1970.) 50s.

EMPIRICAL Bayes methods have been developed to deal with the following situation. An observation x has a probability distribution which depends on an unknown parameter λ about which it is required to estimate or to test hypotheses. Additional observations x_i , similarly dependent on λ_i ($i=1, 2, \dots, n$) are also available, the unknowns λ and λ_i having come from a common unknown distribution, $G(\lambda)$, say. A practical example is quality control where x is a sample value from a batch of quality λ and x_1, x_2, \dots, x_n are similar observations on previous batches. It is clear that estimation of λ might be improved by using experience gained in sampling other batches.

This monograph is the first text devoted to these methods and is welcome on that account. After an introductory chapter there follows one on the estimation of the distribution $G(\lambda)$ —which is not straightforward because the λ s are unobserved and can only be approached by means of the x s—and two on estimation and hypothesis testing respectively. This material is mostly confined to the single parameter case: there then follows a chapter on two-parameter problems. A related situation is the compound decision problem where all the λ s are to be estimated: this is discussed in a final chapter.

Empirical Bayes methods have been hailed as one of the major advances in modern statistics. This is not the place to argue my view that this is incorrect and that the methods are unsatisfactory. Anybody interested in Bayesian methods will learn nothing from this book because an empirical Bayesian eschews Bayesian methods entirely.

The text covers the material adequately. Particularly valuable features are the wealth of illustrative examples and the detailed computational results. A fair number of errors remain and there are some defects of exposition, but these do not seriously disturb a valuable contribution to this curious corner of statistics.

D. V. LINDLEY

Correspondence

Dangers in the Use of the Science Citation Index

SIR,—Attention has been drawn (see *Nature*, **227**, 669; 1970) to the usefulness of the *Science Citation Index (SCI)* for studying science. Certainly, as the author makes clear, this is likely to be a valuable tool for the study of the history and development of science.

However, there appear to be certain dangers inherent in applying it to some of the potential uses described in the paper. For example, once the uses of the *SCI* become more widely known and accepted, an uncritical non-specialist committee seeking to appoint a specialist to a post may well use a diagram of the form shown in Fig. 1 of the paper to appoint the author of paper 2 without going through the tiresome business of reading papers 3 to 15 in detail. Not all citations are complimentary ones, however, and there is nothing in Fig. 1 to show that the author of paper 2 did not make a major "boob" which is subsequently being held up as an unfortunate example by the later authors. It may even have been a clever hoax paper which has subsequently been debunked.

Another disadvantage is the necessarily long time constant involved—the author of paper 2 may have been doing good work in the years before 1947, but the real question in the case of an appointment to a post is what sort of work is he doing or capable of doing more than 13 years later? There is also a danger that the person who is on top of his field in the late 1950s (referring again to Fig. 1 of the paper) might well be automatically passed over because of insufficient time having elapsed for his work to have been formally published and formally recognized, even though fellow workers in his field are already making use of his results. Thus, use of the *SCI* for merit awards may well result in a form of advancement according to seniority.

An interesting anomaly is that it could be possible for a scientist to be so well established that he would drop right out of the "top fifty". How many authors refer to "The Theory of Relativity (Einstein, 1905)" rather than "Einstein's Theory of Relativity", or simply "The Theory of Relativity"?

The author further suggests that "prizes, grants, fellowships and other forms of recognition, could be awarded without the wasteful in-fighting and manoeuvring among scientists described by Watson". Possibly, but it will be interesting to see if extended use of the *SCI* for this purpose leads to a significant decrease in the average number of citations listed per paper. In particular, under this system, detrimental references to another paper could actually assist the author of that paper to win an award.

A further difficulty of a technical nature is that many authors change the style of their name throughout their career. For example A. B. Jones and A. Jones may well be the same person, but in the *SCI* they will receive separate listings. Conversely two different J. Smiths may appear as one person in *SCI*. Moreover, on marriage, many women scientists change to using their husband's surname on their papers, and so will receive separate pre- and post-marriage listings.

Finally, the author ranked as No. 1 in Fig. 3 of the paper is shown as having an outstanding superiority in number of citations over all others. Reference to *SCI* for 1967 shows that this is due largely to a single 1951 publication¹ which is cited approximately 2,350 times. However, because this paper has three other authors, none of whom appear in the top "fifty" of Fig. 3, it would appear that to obtain a good *SCI* rating it is all-important to ensure that one's name appears first on a multi-author paper—or has the computer erred? The paper in question is entitled "Protein Measurement with the Folin Phenol Reagent".

Not being an expert in biochemistry, I am unable to evaluate its worth, though according to the *SCI* criteria it must be one of the outstanding scientific papers of the last few decades. It would be instructive to hear from a biochemist why it is that this particular paper is quoted so prolifically in comparison with all other scientific papers. Dr Garfield clearly recognizes the possibility of abuse of the *SCI*, and he and other experts in the field of science information will undoubtedly be aware of these and other dangers. There does remain, however, the difficulty that the techniques involved are simple enough to be used superficially by uninformed persons.

Yours faithfully,

DAVID L. CROOM

Radio Astronomy Branch,
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Bedford, Massachusetts.

¹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

The Enzyme Defect in Fabry's Disease

SIR,—In your issue of May 16 (*Nature*, **226**, 596; 1970) your correspondent stated that Fabry's disease can now easily be diagnosed because of the absence of ceramide trihexosidase in the plasma of patients with the disease. In fact, the assay of this enzyme which requires the isolation and the purification of ceramide trihexoside is not an easy task for most clinical laboratories, even in university hospitals.

The finding that Fabry's disease is simply due to the absence of a non-specific α -galactosidase is, in this respect, far more promising. This lysosomal enzyme is not detectable in leucocytes, in the plasma and in the urine of male patients with the disease. The α -galactosidase activity in the leucocytes and in the plasma of female carriers of the disease is only 15 to 40 per cent of the amount present in normal leucocytes and plasma. The enzyme determination is quite simple and needs only a colorimeter if one uses *p*-nitrophenyl- α -D-galactopyranoside as substrate or a fluorimeter if the more sensitive assay with 4-methylumbelliferyl- α -D-galactopyranoside is preferred. Both substrates are now commercially available from Koch-Light Laboratories Ltd, Colnbrook, Buckinghamshire, and their distributors in the United States, Pierce Laboratories.

Yours faithfully,

J. A. KINT

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¹ Kint, J. A., *Science*, **167**, 1268 (1970).

Our Medical Biochemistry Correspondent writes: I am afraid I had overlooked Kint's paper on Fabry's disease. His results are certainly interesting, particularly as the linkage in the ceramide trihexoside was thought to be β . His assay is certainly very simple to carry out, but Brady *et al.*¹ have recently pointed out that one needs to be very cautious in identifying enzyme deficiencies using artificial substrates of this type. In two patients with generalized gangliosidosis, there was very little β -galactosidase activity with *p*-nitrophenyl- β -galactoside as substrate but an increased activity when sphingolipids themselves were used as substrates.

¹ *Biochim. Biophys. Acta*, **210**, 193 (1970).

Obituaries

Professor Walter F. Colby

WALTER FRANCIS COLBY, MBE, PhD, died on July 2 at his Washington residence several weeks after suffering a heart attack at his home in Ann Arbor, Michigan. He would have been 90 years old on July 28.

Colby, a physicist and musician, travelled widely in many parts of the world. For many years he was the "talent scout" for the physics department of the University of Michigan, recruiting faculty members from abroad. He was especially active in inviting outstanding lecturers for the famous Michigan Summer Symposium on Physics, which ran from 1923 to 1941. For several great European physicists their summer at Michigan was their first visit to the United States.

After Colby received his AB degree at Michigan in 1901, he taught piano there in the school of music. He went to Vienna in 1902 to study with the great piano teacher Theodore Leschetizky. The lectures of Ludwig Boltzmann, however, turned his attention to physics. Returning to Michigan in 1904, he pursued his studies of physics and obtained his PhD in 1909. He was appointed to the physics faculty and for several years did experimental and theoretical work on the infrared spectra of molecules, a field in which Michigan pioneered under the guidance of the late Harrison M. Randall. This research played an important part in the early development of the quantum theory. In 1912 Colby was on leave in Munich and worked under Arnold Sommerfeld on problems in wave propagation. He spent the following academic year at the Mount Wilson observatory.

During the First World War, Colby did research on aerial photography for the Eastman Kodak Company. In the Second World War he was a key member of the small group investigating the state of German atom bomb research. Though almost 65 years old, he was active in the war zone, sometimes entering places before the allied troops had taken them. His familiarity with languages, roads and laboratories in Europe was of great value in these operations. He was awarded the US Army Medal of Freedom and appointed as an honorary member of the civilian division of the Order of the British Empire.

After the war Colby became the first director of the intelligence division of the US Atomic Energy Commission. At the age of 73 he retired from this post and joined the staff of the National Academy of Sciences to assist with its programme for scholars from overseas. His worldwide contacts were of obvious importance in this function.

Colby was extremely modest and therefore known only to those he met personally; most of these became his friends. It is, however, unfortunate that his modesty prevented him from writing down his memoirs of the early part of his life, for with his death the last link with

physics at the beginning of the twentieth century has vanished.

Sir Archibald Day

VICE-ADMIRAL SIR ARCHIBALD DAY, KBE, CB, DSO, former Hydrographer of the Navy, died at Dover on July 17.

He first went to sea with the Royal Navy in 1914, at the age of fifteen, and served at sea throughout the First World War. He volunteered for the hydrographic surveying specialization in 1920, joining first HMS Endeavour for surveys on the Egyptian coast, and then served continuously in surveying ships. From 1937 he was Superintendent of Charts in the Hydrographic Department, commissioning a new surveying ship, HMS Scott, in February 1939 for surveys off the east coast of England.

In 1940, Day played a major part in organizing the Dunkirk evacuation. During 1943 and 1944 he held the post of Assistant Hydrographer while charts were being prepared for the Sicilian and Normandy landings; later he led a combined surveying and minesweeping flotilla which opened up the Malay and East Indies ports. He was awarded the DSO for his work in clearing and marking the Rangoon River before the assault on the city.

He returned to normal peacetime surveying as Assistant Hydrographer, and, in 1950, took over from Vice-Admiral Sir Guy Wyatt as Hydrographer of the Navy, a post which he held with distinction for five years. Wyatt had realized the importance to the Fleet of studying the oceans as a whole rather than from the purely bathymetric standpoint, and once again seagoing scientists were welcome on board HM surveying ships. This policy was carried forward by Day, largely in collaboration with the Department of Geodesy and Geophysics at Cambridge, including the completion of a modern world-wide Challenger voyage whose scientists were largely engaged in seismic work in the deep oceans. Cooperation between naval surveying officers and military and civilian oceanographers has continued to grow until today it is accepted as routine.

In 1956, Day was appointed coordinator of operations for the International Geophysical Year, a post he occupied in Brussels for three years. Day could well have been a diplomat, for with great charm and tact he nearly always got his own way without offending anyone. These qualities, together with his keen interest in promoting scientific work at sea, fitted him uniquely for his IGY post, and he travelled relentlessly in his efforts to promote harmonious and cooperative international work among marine scientists.

More recently he prepared a detailed and well documented history of the Hydrographic Department from 1795-1919 (HMSO, 1967). This book provides a great wealth of fact about the development of the department for which he worked for much of his life.

Announcements

University News

Dr A. P. McE. Forrest has been appointed to the Regius chair of clinical surgery in the University of Edinburgh, in succession to Sir John Bruce who retires on September 30.

Professor R. M. Shackleton, professor of geology and director of the Research Institute of African Geology, University of Leeds, is to visit the Haile Selassie I University, Addis Ababa, as Royal Society Leverhulme visiting professor.

Dr R. E. Schultes has been appointed professor of biology in **Harvard University**; he will remain curator of economic botany and executive director of the Botanical Museum.

Appointments

Dr A. F. Spilhaus, jun., has been appointed executive director of the **American Geophysical Union** in succession to **Dr Waldo E. Smith** who has retired.

Miscellaneous

The price of the book *Coral Gables Conference on Fundamental Interactions at High Energy* reviewed in *Nature* (227, 975; 1970) is 150s (\$18) and not as stated.

Dr Walter C. McCrone of the McCrone Research Institute has been awarded the **A. A. Benedetti-Pichler memorial award** for 1970 by the American Microchemical Society.

Recommendations for the award of the **Royal Agricultural Society of England research medal** are invited from heads of university departments, research institutes and other organizations. The medal, together with a prize of 100 guineas, is awarded annually for outstanding research work, carried out in the United Kingdom, which has proved or is likely to prove of benefit to agriculture. The purpose of the award is to encourage the younger research worker whose work has as yet received no special recognition. Further information can be obtained from the Secretary, Royal Agricultural Society of England, 35 Belgrave Square, London SW1. No direct application by candidates will be considered.

International Meetings

September 22-24, **Health Problems of Industrial Progress in Developing Countries**, London (Symposium Secretary, Ross Institute, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT).

October 16-18, **Science and Human Values in a Technological Society**, Boston (Society for Social Responsibility, 221 Rock Hill Road, Bala-Cynwyd, Pennsylvania 19004, USA).

October 26-27, **Stable Combustion of Liquid Propellants**, Pasadena (The Combustion Institute, 16902 Bollinger Drive, Pacific Palisades, California 90272, USA).

October 28-29, **Radiation and Isotope Techniques in Civil Engineering**, Brussels (Bureau Eurisotop, 200 rue de la Loi, Berlaymont Building, 1040 Brussels, Belgium).

November 9-11, **Systems Approach and the City**, Cleveland (Professor Arnold Reisman, Systems Research Center, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, USA).

November 19, **Progress in Organic Chemistry**, London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

November 24, **Prostaglandins: Their Possible Physiological, Pathological and Therapeutic Roles**, London (Dr Alma B. Simmonds, Chelsea College, Manresa Road, London SW3).

December 7-12, **Plant Growth Substances**, Canberra (Executive Secretary, Australian Academy of Science, Gordon Street, Canberra City, ACT 2601, Australia).

December 9-11, **Composition and Analysis of Coins**, London (Organizing Secretary, Symposium on Coin Analysis, Ashmolean Museum, Oxford OX1 2PH).

December 14-17, **Structural History of the Bay of Biscay**, Paris (L. Montadert, Division Geologie, Institut Francais du Petrole, BP 18, 92 Rueil Malmaison, Hauts-de-Seine, France).

January 5-7, 1971, **Solid State Physics**, Manchester (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

January 14-15, 1971, **Laser Power and Energy Measurements**, Boulder (Administrative Officer NBS Radio Standards Physics Division, Boulder, Colorado 80302, USA).

March 4, 1971, **Valence in Inorganic Molecules**, London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

March 16-19, 1971, **High Frequency and Microwave Impedance**, Boulder (R. E. Nelson, 272.04 HF Circuit Standards Section, National Bureau of Standards, Boulder, Colorado 80302, USA).

March 30, 1971, **Enzymes in Industry**, Birmingham (R. C. Ashton, Chemical Engineering Department, University of Birmingham, Birmingham 15).

March 30-April 2, 1971, **Fluid Sealing**, Coventry (H. S. Stephens, 5 FICFS, British Hydromechanics Research Association, Cranfield, Bedford).

April 18-24, 1971, **Solvent Extraction**, The Hague (ISEC '71, 14 Belgrave Square, London SW1).

April 26-28, 1971, **Low Frequency Electrical Standards**, Gaithersburg (R. F. Dziuba, Electricity Division, National Bureau of Standards, Washington DC 20234, USA).

May 3-5, 1971, **Colorimetry and Spectrophotometry**, Gaithersburg (I. Nimeroff, NBS Office of Colorimetry, Washington DC 20234, USA).

May 6, 1971, **The Structures of Flexible Molecules**, London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

May 10-13, 1971, **Static Electrification**, London (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

June 2-4, 1971, **Laser Engineering and Applications**, Washington DC (Courtesy Associates, attention Dorothy Edgar, 1629 K Street NW, Washington DC 20006, USA).

July 19-24, 1971, **Continuous Culture**, Oxford (The Organizing Secretary, Continuous Culture Symposium 1971, Society of Chemical Industry, 14 Belgrave Square, London SW1).

August 23-28, 1971, **Microwaves**, Stockholm (Dr H. Steyskal, Secretary General, 1971 European Microwave Conference, Fack 23, 104 50 Stockholm 80, Sweden).

August 26-31, 1971, **Stereology**, Berne (Third International Congress for Stereology, Anatomisches Institut Buhlstrasse 26, CH-3000 Berne, Switzerland).

September 1-3, 1971, **Multivariable Control System Design and Applications**, Manchester (UKAC 1971 Convention Secretariat, Institution of Electrical Engineers, Savoy Place, London WC2).

Sabbatical Itinerants

Entries of this kind can now be found among the classified advertisements

British Diary

Monday, September 14

Aerodynamic Noise (four-day symposium) Loughborough University of Technology, in collaboration with the British Acoustical Society, and the Royal Aeronautical Society, in the Edward Herbert Building, University of Technology, Loughborough.

Photoionization Phenomena and Photoelectron Spectroscopy (three-day conference) Institute of Physics and the Physical Society, Atomic and Molecular Physics Sub-committee, in collaboration with the Theoretical Chemistry Group of the Chemical Society, at the University of Oxford.

The Economics of Ammonia Production (two-day international symposium) Fertilizer Society, at Shell Centre, London SE1.

The Physics of Non-crystalline Solids (third international conference) at the University of Sheffield.

Tuesday, September 15

Advanced Class Boiler Feed Pumps: Application, Design, Development and Operation (three-day convention) Institution of Mechanical Engineers, Fluid Plant and Machinery Group, at 1 Birdcage Walk, London SW1.

Bio-medical Engineering Exhibition (four days) in the West Hall, Olympia, London W14.

Biosynthesis of Branched Chain Sugars, Professor H. Grisebach; also Short General Papers, Carbohydrate Discussion Group, at Bedford College (University of London), Regents Park, London NW1.

Fourth Annual Solid State Devices Conference (four days) Institute of Physics and the Physical Society; the Institution of Electrical Engineers; the Institution of Electronic and Radio Engineers; and the Institute of Electrical and Electronic Engineers, at the University of Exeter.

Gas Discharges (four-day international conference) Institution of Electrical Engineers, in association with the Institute of Physics and the Physical Society, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Wednesday, September 16

Gearing in 1970 (three-day conference) Institution of Mechanical Engineers, at the University of Cambridge.

Growth and Termination Polymerization Processes (two-day symposium) Chemical Society, at the University of Aberdeen.

Modern Machinery for the Manufacture of Paint and Printing Inks (4.30 p.m.) Dr D. O. Sullivan, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1.

Operation and Maintenance of Clean Rooms from the User's Point of View (2.30 p.m. symposium) Society of Environmental Engineers, in the Mechanical Engineering Department, Imperial College, Exhibition Road, London SW7.

Thursday, September 17

Industrial Uses of Radioisotopes (10.30 a.m.) Society for Analytical Chemistry, at the Regional College of Technology, Liverpool.

Friday, September 18

The Nuffield Advanced Physics Course and the Implications for Higher Education (three-day conference) Institute of Physics and the Physical Society, at the University of Birmingham.

Monday, September 21

Electronic Engineering in Ocean Technology (four-day conference) Institution of Electronic and Radio Engineers, and the Institution of Electrical Engineers, at the University College of Swansea, South Wales.

Temperature Measurement (three-day conference) Institute of Physics and the Physical Society, in association with the Institution of Electrical Engineers; the Institution of Electronic and Radio Engineers; and the Institute of Measurement and Control, at the University of Warwick.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Wye College, University of London. Annual Report of the Department of Hop Research, 1st April 1969 to 31st March 1970. Pp. iii+71. (Wye, Near Ashford, Kent: Wye College, 1970.) 8s (40p). [208]

Ministry of Technology and Fire Offices Committee Joint Fire Research Organization. United Kingdom Fire and Loss Statistics 1968. Pp. vi+145. (London: HMSO, 1970.) 24s net. [208]

University Grants Committee. Statistics of Education 1968. Vol. 6: Universities. Pp. xxxvi+151. (Department of Education and Science Series.) London: HMSO, 1970.) 48s net. [208]

Society for Analytical Chemistry. Report of the Analytical Methods Committee, 1969. Pp. 33. (London: Society for Analytical Chemistry, 1970.) [208]

British Museum (Natural History). Publication No. 688: Gilbert White of Selborne. (London: British Museum (Natural History), 1970.) [208]

Proceedings of the Royal Irish Academy. Vol. 69, Section A, No. 7: Some Properties of a Class of Regular Functions. By F. Holland. 3s. Vol. 69, Section A, No. 8: Basic Representations of Finite Semigroups. By D. B. McAllister. 3s 6d. Vol. 70, Section A, Nos. 1 and 2: The Band Spectrum of ZrBr, The Band Spectrum of ZrI. By Ch. Sivaji and P. Tiruvenganna Rao. 5s 6d. Vol. 69, Section B, No. 10: Late-Glacial Vegetational History of Legale, Co. Down. By G. Singh. 8s 6d. Vol. 69, Section B, No. 11: Trace Elements in the Gabbros, Olivines, Augites, and Magnetites of the Carlingford Complex, Ireland. By M. J. Le Bas. 7s 6d. Vol. 70, Section B, No. 1: Alga Lophosiphonia on the Cork Coast. By S. P. Cullinane. 2s (Dublin: Royal Irish Academy, 1970.) [208]

Other Countries

Smithsonian Contributions to Zoology, No. 48: Neotropical Microlepidoptera XVIII—Revision of the Genus *Peleopoda* (Lepidoptera: Oecophoridae). Pp. 30. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office, 1970.) \$0.40. [138]

US Department of Agriculture: Agricultural Research Service. Production Research Report No. 116: Materials Screened as Animal Systemic Insecticides at Kerrville, Texas, 1960-1967. By R. O. Drummond. Pp. 46. (Washington, DC: Government Printing Office, 1970.) \$0.40. [138]

Proceedings of the Second National Science and Engineering Conference, Ottawa, Ontario, January 16, 17, 1970, which established SCITEC. Pp. 94. (Ottawa: Science Council of Canada, 1970.) [138]

Annals of the Transvaal Museum. Vol. 26, No. 7: Neue Laminien aus Südafrika (Coleoptera: Cerambycidae). Von Stephan von Breuning. Pp. 169-176. Vol. 26, No. 8: South African Bark and Timber Beetles. By Karl E. Schedl. Pp. 177-182. Vol. 26, No. 9: Notodontidae Africains Nouveaux, X (Lepidoptera: Notodontidae). Par S. G. Kiriloff. Pp. 183-198. Vol. 26, No. 10: A Re-Examination of some Southern African Scorpion Species (Arachnida: Scorpionidae). By G. Newlands. Pp. 199-210. (Pretoria: Transvaal Museum, 1970.) [138]

Republic of South Africa: Department of Industries. Division of Sea Fisheries Investigational Report No. 88: Marine Algae from Southern Africa. 1: Six New Species from the Inter- and Infra-Tidal Zones. By R. H. Simons. Pp. 13. (Sea Point, Cape Town: Director of Sea Fisheries, 1970.) [138]

US Department of the Interior: Geological Survey. Bulletin 1314-B: Absorption of Gold by Plants. By Hansford T. Shacklette, Hubert W. Lakin, Arthur E. Hubert and Gary C. Curtin. Pp. iii+23. \$0.30. Bulletin 1319-B: Mineral Resources of the Jack Creek Basin, Madison County, Montana. By George E. Becraft, Thor H. Killgaard and Ronald M. van Noy. Pp. vi+24+2 plates. \$0.65. Water-Supply Paper 1962: Quality of Surface Waters of the United States 1965. Parts 3 and 4: Ohio River Basin and St. Lawrence River Basin. Pp. xi+480. \$2.25. Professional Paper 313-E: Mineral Resources in Permian Rocks of Southwest Montana. By Roger W. Swanson. Pp. v+661-777+4 plates. (Washington, DC: Government Printing Office, 1970.) [138]

US Department of Commerce: Bureau of the Census. State Government Finances in 1969. Pp. 59. (Washington, DC: Government Printing Office, 1970.) \$0.60. [148]

Molecules, Medicines and You. Pp. 16. (Washington, DC: Pharmaceutical Manufacturers Association, 1970.) [148]

World Health Organization. Control of Pesticides: A Survey of Existing Legislation. Pp. 150. (Geneva: WHO; London: HMSO, 1970.) 12 Sw. francs; 24s; \$4. [148]

United States Naval Observatory. Circular 127: Observations of the Sun, Moon, and Planets—Sixth-Inch Transit Circle Results. By B. L. Klock and D. K. Scott. Pp. 13. Circular No. 128: Astronomical Data in Machine Readable Form. By Solomon Elvove. Pp. 11. (Washington, DC: United States Naval Observatory, 1970.) [148]

National Institute of Child Health and Human Development, Bethesda, Md. Key Issues in Infant Mortality—Report of a Conference, April 16-18, 1969, Washington, D.C. Scientific Editor: Frank Falkner. Pp. vii+84. (Washington, DC: Government Printing Office, 1970.) \$1. [148]

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Clear Voice from the East

THE first thing to be said about the extract from Dr Zhores Medvedev's latest book which appears on page 1197 is that this moving tale rings true. Over the years, the difficulties of inviting Russian scientists to conferences in the West have become familiar to academics both in Europe and the United States. A part of the difficulty is that the mails are slow to travel but there are also serious impediments on the Russian side and within the Russian bureaucracy. Dr Medvedev has a great many other tales to tell of how he and his colleagues have been frustrated in their attempts to keep links with scientific work in the West by ignorance, malevolence or sometimes mere hesitancy in the agencies of the Soviet Government. His book as a whole is a plea addressed not so much to the English-speaking audience that it will reach but to the Russian hierarchy where changes are most urgently needed. In short, Dr Medvedev has written a constructive criticism of the present arrangements and not a piece of subversive literature. It is earnestly to be hoped that it will be so regarded in the Soviet Union and in the West.

The second thing to say is that Dr Medvedev is clearly a remarkable man in the best tradition of all the ordinary people who have over the years spoken up against the suppression or the erosion of personal liberty. The circumstances of his case are now well known. Early in the sixties, he was commissioned by the Soviet Academy of Sciences to write an account of the doctrine of genetics according to Lysenko, but this tale was never published in the Soviet Union because Medvedev sought to deal not merely with the ways in which Lysenko was mistaken but also with the circumstances which had allowed his false doctrine to flourish in the Soviet Union without serious protest from scientific institutions such as the academy. The appearance of his book (*The Rise and Fall of T. D. Lysenko*, Columbia University Press, 1969) was important for the light it threw on the workings of the Russian scientific establishment. For Dr Medvedev himself, the occasion was important and also damaging—soon afterwards, he lost his job at the institute at which he had worked for several years. In May this year, Dr Medvedev was taken from his house and kept in a psychiatric institution until the protests of his friends and colleagues, many of them the most distinguished scientists in the Soviet Union, led to his release in June. Throughout these trials, however, Dr Medvedev has not stifled his protest against a system which is not only unjust but, in a curious way, more seriously in error because it is also wasteful of people's talent. Yet nothing in what Medvedev has written suggests disloyalty to or even disagreement with the political foundations on which the Soviet Union is based. He is not a revolutionary or

a counter-revolutionary but, rather, a heroic but frustrated reformer.

What is the complaint? Why is it important that distinguished scientists who happen not to be a part of the Soviet hierarchy, rank and file scientists striving to make a reputation and even young scientists embarking on their careers should be free to accept invitations to lecture abroad? Why should scientists outside the Soviet Union worry if Russian scientists are chained to their laboratory benches or otherwise prevented from learning what happens elsewhere? And is it really as damaging as Medvedev says that the scientific community in Russia is left with the haunting sense of isolation that pervades his book? Are not monasteries good places for contemplation? The trouble, of course, is that the scientific community is indivisible. The old slogans about the international character of the scientific enterprise are, strange to tell, true. Medvedev's book is an often painful account of how the efforts of good people are so frustrated by artificial circumstances that a potentially important contribu-

***Nature* Three Times a Week**

FROM the beginning of 1971, *Nature* will appear three times a week, on Monday, Wednesday and Friday. As at present, the Friday issue will contain news of general interest within the scientific professions, notes on important developments in science, review articles, book reviews and original articles. From January next, the Friday issue of *Nature* will also include enough information about the contents of the Monday and Wednesday issues for weekend readers to be fully informed of the somewhat specialized material to be found in the Monday and Wednesday issues of *Nature*. This development is intended to provide those who look to *Nature* for a continuing record of the development of science with a fuller account than is possible within the confines of a single issue every week. At the same time, *Nature* should become an even more effective vehicle for the publication of original research.

Further details will be announced towards the end of October, but from January 1971 unexpired portions of subscriptions to *Nature* will be satisfied for an appropriate period by copies of the weekend *Nature*, the subscription price of which will not be greater than the present price of *Nature*.

See also leading article, page 1179.

tion to understanding is lost for good, and even when tangible impediments to creative work do not exist, there is always the haunting fear that isolation from the outside world, exemplified most clearly by the lack of scientific journals, will rob a scientist of the confidence he needs to tackle something new. Ascetic it may be, but the nature of scientific work requires that innovations must be set against the background of known discovery if they are to be meaningful. There is a sense in which each scientific discovery of importance is an achievement of the scientific community as a whole. The malice and bumbledom which isolate Russian scientists from the outside world deprive the scientific community as a whole of the contributions of a host of talented but frustrated people; they are a cruel deprivation of the spirit for those inside the Soviet Union. This is plain from Medvedev's account.

There is much that can usefully be done. As things are, the arrangements for the exchange of people between the Soviet Union and countries such as Britain and the United States are regulated by bilateral agreements. This is what Dr Medvedev means when he talks of the limit on the number of man-days per year for which Soviet scientists may be absent from the Soviet Union. To be sure, there has been a steady growth of this valuable traffic in people as the years have gone by and the prospect of still further expansion is, of course, enormously to be welcomed. Yet bilateral agreements remain needlessly formal in character. The notion that an application for permission to visit a foreign country should be made by the academies to which the people concerned belong is foreign to the tradition that in scientific work, people should be helped to come and go as they please. And under the present arrangements it is often hard for would-be hosts in countries outside the Soviet Union to ensure that an invitation will be taken up by the most suitable person—often a colleague in some related field is offered instead. The result is that the system of exchanges now in service makes less than full use of the opportu-

ities which present themselves when people working on the same problem are able by collaboration to stimulate each other beyond the bounds of ordinary expectation. In short, the most desirable reform of the present system of exchanges is not mere growth, valuable though that would be, but a more liberal and flexible policy that would allow people to come and go with much less formality than is at present the case. It is true that such a development would conflict with the Soviet Government's attitude towards its nationals, and in that sense Dr Medvedev is asking for a quite radical reform. Even from the Soviet Government's point of view, however, the risks involved would be far outweighed by the potential benefits of a closer connexion between the Soviet community of scientists and their colleagues elsewhere. The record of the past two hundred years in Europe shows this to be the case.

But is it reasonable to hope that Dr Medvedev's book will be a catalyst for such far-reaching changes? Nobody can be sure. What is, however, beyond dispute is that Dr Medvedev has been fearless in his advocacy of his cause and that his courage has already shamed many estimable scientists in the Soviet Union to take up the same theme. This is why the publication of his book—the manuscript of which found its way to *Nature* in much the way in which some manuscripts describing original research arrive—must therefore not be considered a personal disservice to the author. There are risks, it is true, but Dr Medvedev seems well aware of them. His friends in the West consider that on balance publication is what he would wish. One of the ironies of the situation, explained in his book, is that the photographic copies of *Nature* that circulate in the Soviet Union will almost certainly lack all references to this text. That is a small but telling reminder of the isolation of Soviet scientists. The only certainty is that sooner or later this sense of isolation must be attenuated. Long before then, Dr Medvedev will know how important a part he has played in an important cause.

Will the Thames Flood?

PROFESSOR A. N. SCHOFIELD's calculation (page 1203) of the likelihood that it would now be profitable to ensure against the risk of flood damage around London by building lines of sandbags alongside the river will be—or should be—a sombre reminder of how even great cities such as London can live uncomfortably close to disaster. The mean level of high water in the London river is rising at the rate of 2.5 feet a century and, as it does so, the frequency of the occasions when the river defences are breached must necessarily increase. The way things are going, there could be a serious flood each decade in the years ahead and the burden of Professor Schofield's argument is that prudent insurance against this risk would justify the expenditure of something of the order of £100 million. How is this to be accomplished? Professor Schofield argues that sandbagging the embankments

would be the cheapest insurance policy and that it makes economic sense to undertake a programme of that kind as a matter of urgency. The other more expensive ways of controlling flooding in the river—the barrage across the estuary—are, he argues, not merely expensive forms of insurance but slow to bring into operation.

What will the planners make of this? And how will those who worry about remote dangers such as the possibility that the accumulation of carbon dioxide in the atmosphere may affect the climate a century from now respond to the suggestion that it would be worthwhile to spend more than £100 million on sandbags along the Thames so as to fend off a much more immediate danger? The chances are that there will be comparatively little enthusiasm for a defence of London against the Thames, at least until there has been a serious flood. Even then, it is more than likely

that people will misinterpret the statistics in such a way as to assume that serious flooding cannot again occur for another decade. The truth is, however, that Professor Schofield is almost certainly correct in urging that steps should urgently be taken to contain the

Thames even if there may be room for doubt about the statistics on which his calculation of the frequency of flooding is based and even though there is no doubt that a line of sandbags along the river would be the most inelegant defence conceivable.

Another Journal Proliferates

PLANS for increasing the volume of the scientific literature are now customarily regarded by scientists as offences against the proper conduct of business, which in turn implies that those seeking to increase the volume of the literature should offer some kind of explanation, excuse or even apology. Where the plan for publishing *Nature* three times a week is concerned (see page 1177), the freedom which the new scheme will provide for satisfying the original objectives of the journal is a sufficient explanation. To begin with, 101 years ago, *Nature* was at once a means of reporting the results of original research and a means of informing professional scientists about important developments in science—the publication of books, the proceedings of scientific societies and the doings (usually nefarious) of governments. A mere forty pages of text each week were usually enough to keep people properly informed. Over the years, however, the contradictions between these objectives have been made often painfully apparent by the growth of scientific activity. In the past few years, one of the most serious problems has been that of reconciling the interests of specialists that original work should be reported rigorously and in some detail with the interests of the general readers of the journal (mostly specialists in their own right) that much of *Nature* should be widely intelligible. One result has been that the demand for space for original research has complicated the task of providing a continuous and comprehensive record of what happens in and about science. Another has been that in the process of selecting a tiny proportion of original manuscripts from the flood arriving with every post, *Nature* has often seemed to its contributors to be arbitrary and even fickle in making decisions.

The new pattern of publication, to begin in the new year, should help to reduce these pressures in several important ways. First, there should be more room each week for original research, which will be distributed throughout the three editions. Second, there should be more room for news of important developments in science, especially at the weekend. Third, it will be possible to provide in the weekend journal information for non-specialists about a greater variety of original material appearing elsewhere, either in *Nature* or in other scientific journals. Fourth, it should be possible to broaden the scope of the weekend journal so as to deal with some important matters now unwillingly neglected. It would be wrong, however, to expect that the weekday editions of *Nature*—those published on Monday and Wednesday—will be ragbags into which are thrown reports of original

research which are too indigestible for a general readership. On the contrary, these editions will make it possible to report news which is in many ways too specialized to find its way into print at present as well as technical reviews of a kind which may seem especially appropriate in a journal which appears frequently and which can aim at being topical. In other words, although the weekend journal will have the broadest theme, the other two editions will be put together so as to make a journal which is pleasurable to read. This has always been one of the guiding principles of weekly journalism and of *Nature* and there is no intention that it should now be abandoned.

100 Years Ago



PROFESSOR HUXLEY's presidential address is not his only outcome at Liverpool which it is our duty to chronicle—a duty which we perform with gratitude to him for his plain speaking. At the unveiling of Mr. Gladstone's statue on the 14th inst., Mr. Huxley, after referring to the Compulsory Education measure, which promises in time to rid us of our worse than Eastern degradation, as one of Mr. Gladstone's greatest achievements, added that if he might presume to give advice to a man so eminent as Mr. Gladstone—if he might ask him to raise to a still higher point the lustre which would hereafter surround his name in the annals of the country, it was that he should recollect there was more than one sort of learning, and that the one sort which was more particularly competent to cause the development of the great interests of the country, was that learning which we were in the habit of calling Science. That Mr. Gladstone was profoundly acquainted with literature, that he was an acute and elegant scholar, they all knew, but he suspected that the full importance for the practical interests of the country of developing what was known as Science was not quite so clear to the Prime Minister as it might be. But, seeing the great faculty of development which his past career had shown, he had no doubt that such a man would by-and-by see that if this great country was to become what it should be, he must not only put the means of education within the reach of every person in the land, but must take care that the education was of such a nature as to provide those persons with the knowledge which they could apply to their pursuits, and which would tend to make them understand best those laws under which the human family existed.

From Nature, 2, 414, September 22, 1870.

OLD WORLD

ACADEMIC FREEDOM

University Teachers Unite

THE waves of indignation which spread through some British universities over the so-called Atkinson affair have not yet spent all their energy. A conference to establish a Council for Academic Freedom, linked with the National Council for Civil Liberties, will be held at Imperial College, London on October 3. The aims of the proposed organization are to protect the rights of academic staff to express political opinions without fear of academic reprisals, and it has clearly been prompted by the decision of the academic appointments board at the University of Birmingham to reject the nomination of Mr Dick Atkinson for reasons which Mr Atkinson's supporters claim are not entirely academic. Mr Atkinson, a lecturer in sociology at the University of Manchester, applied for a post at Birmingham last July.

The conference on October 3 will have before it seven proposals, including a statement of the aims of the proposed council "to defend and promote academic freedom in universities and all other institutions of higher education, to inform its members of cases where academic freedom has been breached or is threatened, to take whatever action is appropriate in such cases, and to promote the publication of other information related to academic freedom". Invitations to take part in the conference have been sent out to some 500 academic staff, and Professor Norman Dorsen, a member of the General Council of the American Civil Liberties Union, has been invited to speak.

The council will function under the auspices of the National Council for Civil Liberties, although it will be autonomous as far as policy making is concerned. The idea clearly is to use the machinery of the NCCL—particularly the NCCL's lawyers—to operate the council and in return the NCCL will gain an influx of new members. Members of the Council for Academic Freedom will therefore have to pay the usual membership fee for the NCCL, in addition to an annual fee of 10s.

The council will be run by a committee consisting of ten members elected by the conference and three members nominated by the NCCL, of whom one will be the general secretary, Mr Tony Smythe. Membership will consist chiefly of teaching staff in higher education, but it will also be open to "other interested persons".

The Association of University Teachers, which in the past has been the body chiefly concerned with the rights of university teaching staff, is lukewarm about the proposed council. Mr Laurie Sapper, general secretary of the AUT, said that "if the people who are hoping to set this organization up were willing to put as much energy into making the AUT work more effectively, there would be no need for such a body". Mr Sapper also pointed out that last year the AUT spent £2,000 on legal costs incurred in fighting the dismissal of three lecturers, one of whom was Mr Robin Blackburn who was dismissed by the London School of Economics for his part in the disturbances there.

On the other hand, Professor John Griffith, of the London School of Economics—one of the prime

movers behind the proposed council—believes that the "AUT's heart is in the right place, but it doesn't actually do very much". The council will be anxious, however, to cooperate with the AUT, the Association of Teachers in Technical Institutions and the National Union of Students. Other sponsors of the council include Professor David Epstein of the University of Warwick, Professor David Glass of LSE, Professor Peter Townsend of the University of Essex and Professor Peter Worsley of the University of Manchester.

RESEARCH COUNCILS

NERC Grows On

THE report of the Natural Environment Research Council for the year ending on March 31 (HMSO, 13s) is a cheerful record of growth on the rising part of a sigmoid curve. The council estimates that in the past year, it spent £11.84 million. As always, the Natural Environment Research Council chooses to spend a high proportion of its resources on the organizations such as the Nature Conservancy which it inherited at its inception or which it has since set up on its own. Research grants and training awards account for only £1.84 million, just over 15 per cent of the total. Although a great deal of this imbalance may be explained by the high cost of operating the common facilities such as research vessels, it will no doubt be a considerable disappointment to academics that only £1.25 million found its way into universities. In the year just past, the luckiest universities were Cambridge (£0.11 million) and Imperial College, London (£0.096 million). At the other end of the scale, the University of Surrey was granted £877 for research and Chelsea College of Science and Technology less than half of that.

The council's report says that there has been a "considerable evolution" in its policy towards post-graduate research at the universities. What seems to be implied is that the council is trying to develop a strategy for research based on the collective views of university departments about the directions most likely to be profitable. The report says that the council plans to increase its allocation of funds to university research "if the flow of applications for worthwhile projects is sustained" by about 6 per cent a year.

Pollution and the environment are two of the matters on which the council is concerned. The report says that the council has in mind a long term programme of research consisting of systematic ecological surveys intended to throw up evidence of changes brought about by pollutants, experimental studies of the biological effects of specific pollutants, especially at chronic doses, and research on the persistence and degradation of biological contaminants.

ROCKETRY

European Space after Apollo

from a Correspondent

LORD BESSBOROUGH, Minister of State at the Ministry of Technology with responsibility for aerospace matters, is leading the British representation that is taking part in the European Space Conference (ESC) mission to

Washington. The week of talks to establish whether the United States is prepared to supply launchers for European applications satellites during the 1970s, and if so, what the political and financial terms are going to be, opened on September 16. The talks are also meant to explore the implications of European participation in the American post-Apollo programme that will come into action during the 1980s. A great deal of American diplomatic and industrial pressure has been exerted on Europe during the past nine months without achieving a clear response. The key question at this stage would seem to be whether a definite European commitment at government level to support the post-Apollo developments is a condition of American undertakings to supply conventional launchers for European aspirations in the meantime.

If credible and positive reassurances can be obtained, there would seem to be no remaining justification for pursuing further the Europa launcher project, lagging in its development schedule with continually increasing costs, on which crucial decisions are to be made at the European Space Conference at Brussels in November.

The ESC mission is led by M. Theo Lefèvre, the Belgian minister responsible for science and chairman of the European Space Conference. The inclusion of senior British and French members was at the request of Germany when the mission was proposed in July. There seem to have been difficulties, however, in gaining the participation of M. F.-X. Ortoli, the French minister responsible for science. It is not expected that all the knotty problems at issue will be fully clarified in one week of talks, so that a further visit in the autumn is likely. Although the continuation of July's European Space Conference is scheduled for early November, the mission is not due to report back until December. Realists are probably not far wrong in supposing that the attitude of the European governments to participation in the post-Apollo programme will not be settled before next spring.

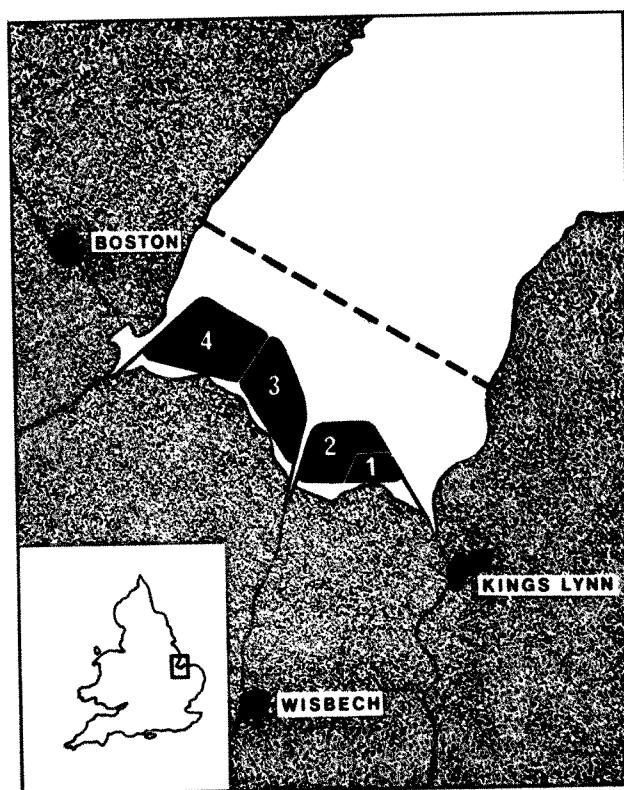
In the meantime, the chief American companies involved in the programme are hurrying on with the selection of partners for the design phase, in particular of the space shuttle element of the programme. The most recent and definite of the arrangements is the announcement by the British Aircraft Corporation (BAC) during Farnborough week that it has undertaken to carry out about £100,000 to £200,000 of work on the second phase of the shuttle design study being handled by North American Rockwell, due to be completed in June 1971. A small team from Britain alongside the American design team in California will do work "integrated across the board", according to Dr Handel Davies, technical director of BAC. This will specifically include structure, materials, data recovery and the total avionics system. Experience with Concorde and in helping to build one of the Intelsat 4 communications satellites weighed heavily with the American firm in making the selection. A point made by Dr Davies is that over six months BAC has swung from almost total disbelief in the feasibility of the project to complete confidence. Technical assessment of the project has so far been noticeably lacking—the kindest remark is that American presentations have been "woolly"—so it must be hoped that BAC has done its homework and has not been carried away by anxiety to get in on the ground floor of a new technology.

BARRAGES

Boost for the Wash

THE possibility of using part or all of the Wash, the 300 square mile inlet of the sea on the east coast of England, for the storage of freshwater behind a barrage or in separate offshore reservoirs has been in the air for a long time. But until last week's favourable report from the Water Resources Board (*The Wash: Estuary Storage*, HMSO, £1 1s), plans have been little more than wishful thinking. The report is based on a desk study commissioned by the board in October 1968 from a firm of consulting engineers. Although a barrage enclosing the whole of the Wash has now been shown by the engineers to be impossible technically, and one across the inner half of the estuary is considered unacceptable to the board on other grounds, the board says that it would be feasible to have three or four "bunded" reservoirs, completely enclosed by an artificial bank or bund, about a mile off the present margin of reclaimed land on the south-west shore. The reservoirs could be filled by tunnel or pipeline from points on the rivers Great Ouse, Nene, Witham and Welland, just upstream of the ports.

Many more studies are needed, however, before the details of the scheme can be drawn up, and the board is therefore asking the Government to authorize a full feasibility study which could begin next year and be completed by 1977-78 at a cost of about £1.1 million. But the start of the scheme should not necessarily be held up until the feasibility study is completed, says the board. The study should be so designed that in 1973-74 a decision could be made



The proposed scheme of pumped storage reservoirs in the Wash. The dotted line marks the possible site of a barrage.

whether to build the first (stage 1) reservoir in time to produce water in the early 1980s when the central area of south-east England will need a major new source of supply. The best position for this reservoir would be to the west of the outfall of the Great Ouse; it would cost about £23-£25 million to build, would hold about 14,000 million gallons of water and it could provide up to 100 million gallons a day, depending on the flow in the river.

The reservoirs together would cover about 15 per cent of the total area of the Wash (about 100 square kilometres), and would be at a height of about 9 metres above sea level. But after preliminary consultations with the Natural Environment Research Council, the river authorities and other interested bodies, the board considers that banded reservoirs would have very little effect in the long term on the environment of the Wash. This aspect and more detailed costings and technical details would, however, be investigated more fully in the feasibility study.

The Wash scheme for estuarial storage of water is not the first in Britain. Preliminary studies of the possibilities of estuary storage in Morecambe Bay, the Dee Estuary and the Solway Firth were published in 1966-67, and reports of the full feasibility studies of the first two are expected next year. The board has, however, ruled out a barrage across the Solway Firth, at least in the foreseeable future.

EXHIBITIONS

Bio-medical Engineering

"The advancement of medicine is the most exciting—and in the long term the most important—of all the prospects opened up by the contemporary explosion of knowledge in science and technology." With these words, and with obvious enthusiasm for applying engineering techniques to medicine, Dr J. M. Lenihan, director of the department of clinical physics and bio-engineering, Western Region Hospital Board, Glasgow, set the scene for the Bio-medical Engineering Exhibition held in London this week. Dr Lenihan's enthusiasm is certainly justified by the impressive display of sophisticated medical equipment, and interest shown by the medical profession in the exhibition—the sponsors expect at least 6,000 visitors—leaves little doubt about the commercial attraction in manufacturing specialized medical equipment.

Among the techniques aired at the exhibition, the measurement of deep body temperatures and its use for diagnosing and pinpointing disorders such as thrombosis, varicose veins and breast tumours was prominent. The technique of thermal imaging—picking up infrared radiation from the human body and displaying the resulting thermal image on an oscilloscope—is already firmly established, and it has been used to map the flow of warm arterial blood into, for example, tumours and varicose veins. But research being carried out at the UKAEA Atomic Weapons Research Establishment at Aldermaston may help to speed up the application of the technique. What the Aldermaston team has done is to feed signals from an infrared detector directly into a temperature-calibrated readout system which effectively converts the instrument into a fast-scanning thermometer. An

area 41 cm × 30 cm can be scanned in one second by this machine.

An instrument which could prove valuable for psychological research, a miniature transmitter for EEG, ECG and EMG measurements, has been developed by Smith and Nephew Research Ltd. The transmitter, which measures only 16 mm long, 13 mm wide and 5 mm deep, has already been tried out by Dr G. K. Wallace of the University of Reading. The chief attraction of the new transmitter is that it is much less bulky, and therefore more unobtrusive, than conventional transmitters, and it can be used for research on animals and children without itself affecting the results.

Among the exhibits which are already commercially available, cardiac resuscitation equipment caught the eye and a pneumatic blanket which gives automatic warning of cessation of breathing in premature babies was impressively ingenious.

SUBMERSIBLES

Diving with NERC

IMPRESSED with American advances in the design of submersibles, the Natural Environment Research Council hired a two-man craft from Vickers Ltd for a week last November, to see how useful it might be in marine geology, oceanography, ecology and fisheries research. The submersible, *Pisces II*, was sent to Scotland with its support vessel and went through an intensive series of dives in the open sea off Oban and also in Loch Fyne, a long inlet further south. There were teething troubles, engine failures and even difficulties in recovering *Pisces* from the sea bed, but now that the NERC scientists have reported on their impressions of the week (*NERC publ. series C, No. 2, 1970*), the advantages of exploring the underwater environment at first hand seem in general to have outweighed the snags.

The manoeuvrability of *Pisces* was valuable, and although some of its functions could be replaced by underwater television and sampling from the surface, phenomena on a small scale could be studied in detail in relative comfort. One particular success was in exploring the floor of Loch Fyne, when it became clear that the manganese nodules found in samples obtained by dredging are in fact exposed on the loch bed, and that shell fragments found there represent a living fauna and are not transported debris.

A project to study sea currents by following trails of dye revealed some of the limitations of *Pisces*. On the way down it lost track of the blocks of dye which had been released beforehand, and while manoeuvring at the bottom to look for the lost dye, it churned up so much mud that nothing at all was visible from the ports. The oceanographers felt that apart from search and recovery operations, the value of a submersible is limited. Its potential in marine geology and ecology is considerable, however. Scientists and their equipment can be brought in close contact with an environment that could previously be explored only from the surface, and sampling can be achieved safely and efficiently.

A further series of twenty dives was carried out in June this year at the edge of the continental shelf by the Outer Hebrides under the aegis of the Institute of

Geological Sciences and the National Institute of Oceanography. Pisces performed excellently, reaching a depth of 1,250 feet, which is deeper than anywhere on our continental shelf. A makeshift drill was used to take core samples and external cameras shot some excellent film and videotape. There is no doubt that Pisces will become increasingly useful as more sophisticated tools are developed, of the type already in use on American submersibles. Its use by institutions in Britain is expected to become a regular occurrence.

APPLIED PSYCHOLOGY

Watching Men at Work

WHAT colours are best for postage stamps? How many television screens can be monitored at once? How much noise can be tolerated on a telephone line? These are some of the questions which are being answered at the Medical Research Council Applied Psychology Research Unit in Cambridge, which put some of its work on show last week. The staff of the unit tackle problems set by clients who can be food manufacturers or the army and navy, and they pride themselves for having saved money and effort on many occasions. At the same time the information that their labours accumulate is contributing knowledge about how the human brain works.

The problems facing somebody who has to scrutinize several television screens simultaneously are being investigated on behalf of the Home Office. Television is used for surveillance in several British prisons, and if the full potential of the system is to be exploited certain questions must be answered. For example, what is the largest number of screens that can be watched; what size should they be and for how long can they be watched before the viewer becomes bored or inefficient. To answer these and other questions, Mr A. H. Tickner has made sixteen one-hour films inside and outside prisons. Subjects watch them all simultaneously on small screens arranged like a block of television sets, and are asked to pick out certain incidents which were specially staged to look suspicious in a prison context. So far only thirty-six subjects have been tested, and Mr Tickner cannot say whether any trends are emerging, but it seems possible that the efficiency with which the suspicious incidents are recorded could vary with sex and age of the observer.

A task recently completed by Dr I. D. Brown and Mrs A. J. Hull has been to advise the Post Office on the best colours to use for fourteen low value decimal stamps. Discrimination tests revealed that sorters identified stamps most easily if seven colours were used each at two distinct levels of saturation, for example, dark blue and light blue, dark green and light green. If colours are used purely to produce stamps which are aesthetically pleasing there may well be difficulties in distinguishing one value from another at a quick glance.

The Post Office has another problem with noisy telephone lines; when pulse code modulation comes into regular use it will be very expensive to remove all background noise, and so the engineers would like to know how much subscribers ought to be able to tolerate. Dr C. M. Holloway's experiments have shown that if a line is so noisy that numbers can only just be identified they are hard to remember.

MEDICINE

Hope for Flu Victims

As winter approaches, it is comforting to know that there is promise of a new drug, called amantidine, which may be able to prevent and even to cure influenza. Meanwhile the search for an effective vaccine progresses, and the Department of Medical Microbiology at the University of Liverpool has just received a grant of £6,348 from the Medical Research Council to continue research, directed by Dr D. Dobson, into methods of immunization.

Amantidine has been on trial for nearly ten years but only recently has there been any reliable indication of its ability to protect against the influenza virus, particularly the A2 virus which is active at present. In *The Practitioner* this month there is a report of a small but significant trial conducted during the epidemic of Hong Kong influenza last year in a town in Surrey when nearly a quarter of the population of 5,500 was affected by influenza. Of twenty-two influenza patients treated with amantidine, all responded with a diminution of malaise and headache and twelve returned to a normal temperature the day after the treatment was begun. The results were better than any that could have been expected with other available treatments. Ten contacts were also given the drug as a prophylactic, and none developed influenza. Another trial in Rumania, reported in the *Bulletin of the World Health Organization*, showed the preventive possibilities of the drug. During an epidemic also caused by the A2/Hong Kong influenza virus, 112 people were treated with amantidine daily for twenty days and 103 were given inert tablets. Of those given amantidine, only two developed influenza, compared with twenty in the control group.

The way in which amantidine works is not clear. It does not kill the virus, but it may prevent it from entering the cell. Whatever the mechanism, if future studies confirm the therapeutic value of amantidine, it could become a considerably more flexible control of influenza than any vaccine. Whether or not it can cope with the new strains of virus as they appear, however, remains to be seen.

There are additional problems to be overcome before an effective vaccine can be developed. Of all the antibodies produced in response to influenza infection, it is not known which is essential for protection; the role of the serum antibody in protecting the body against influenza has not been completely defined. The other problem is that the immune response by the host to the virus is relatively poor. This is because the virus enters the respiratory passages directly and the infection remains localized there, separate from the antibodies circulating in the bloodstream. It is now believed, however, that a different type of antibody (of the immunoglobulin A variety) is made in the respiratory epithelium itself and is responsible for a more direct combat with the infection. This antibody is of great interest to the team at the University of Liverpool. They are concerned with finding out which of the several different kinds of antibody is the most effective in giving protection and with determining which is the best form of purified vaccine to produce the right amount of the right kind of antibody in the right part of the body before the epidemic season begins.

Austrian and Dutch Education under Examination

AUSTRIA and the Netherlands have recently had their education policies laid bare by teams of examiners from the Organization for Economic Cooperation and Development. These examinations, although founded on facts which are now rather out of date, highlight many of the problems currently being faced by other industrial countries whose education systems have grown in a haphazard manner and who are now attempting to supply industry with enough trained manpower, while at the same time trying to give every child the same opportunity, regardless of his economic or social background (*OECD Reviews of National Policies for Education: Austria*, HMSO, 11s; *OECD Reviews of National Policies for Education: Netherlands*, HMSO, 15s).

One of the chief and inescapable factors influencing the development of education in Austria is the difficulty of transportation in mountain regions, where schools are often very small. The primary education sector has borne the full effects of this situation, which has given rise to regional disparities in educational opportunity. At the age of ten, pupils either remain at primary schools or transfer to grammar schools or vocational schools. For those who remain, there are essentially two types of education—either they continue to be taught by one teacher for all subjects, or they are taught by specialized teachers. The latter system provides the better education, but it is only practical in large schools and pupils in small primary schools in the mountains therefore often tend to miss out on opportunities to transfer to grammar schools or to go to university.

It is in the grammar schools, which select their students by entrance examination, that the chief expansion has taken place during the past few years. The OECD examining team points out, however, that not only are grammar school students predominantly middle class, but that the system has several other weaknesses. For one thing, there is seldom any transfer of pupils from other schools to grammar schools and educational choices made at age ten therefore tend to dictate a child's future to an unhealthy extent. Professor Westley, a member of the OECD examining team, pointed out during the so-called "confrontation meeting"—a discussion between the examining team and the education authorities—that this situation at least calls for adequate parental and pupil guidance when educational choices are made. Dr L. Wohlgenuth, of the Austrian Ministry of Education, defended the extension of the grammar school system on the grounds that it is preferred by parents.

When it came to looking at the universities, the OECD examiners seemed to be on familiar ground. Their analysis of the Austrian higher education system is both more searching and more specific than their analysis of other parts of the education system. They single out five points of weakness. Firstly, 20 per cent of students in Austrian universities come from outside Austria. This, the examiners suggest, tends to inflate the higher education budget to an undesirable extent. A second area which "would appear to warrant examination" is the length of degree courses. The actual study time is at least one third longer than the average time

spent at universities in most countries. Financial aid to university students should also be put at a realistic level, the examiners suggest, to remove the need for many students to take employment during their studies, and a closer check should be kept on their academic progress, especially by introducing more examinations and other tests.

Finally, a factor which makes the production of graduates an expensive process for the Austrian economy is that many graduates leave the country soon after qualifying. A conservative estimate has put the proportion of engineers who emigrate at about 10 per cent. The examiners, however, do not offer any solutions to the problem of the brain drain.

The Netherlands, like Austria, has a tripartite secondary education system which gives a distinct advantage to children from middle class backgrounds. The OECD examiners point out that "the historical trend of the distribution of pupils among the various types of schools in the Netherlands shows the distribution to be determined more by social class than by the ability of the child". Again, the examiners noted the lack of opportunity to transfer from one type of secondary school to another, and, despite the device of a "bridge year" to stream students in secondary education, the examiners were not satisfied that selection procedures are sufficiently objective. The bridge year, however, "is a great improvement on such drastic selection methods as the original English '11+ examination'".

Mr J. H. Groesheide, State Secretary, Ministry of Education and Sciences, told the OECD examiners at the confrontation meeting that the concepts embodied in the Dutch education act of 1962 do not preclude "nor do they infer the necessity" to establish comprehensive schools. He predicted, however, that many school boards will soon take the opportunity to establish such schools, because there is a growing interest in comprehensive education in the Netherlands.

In the Netherlands, as in most industrialized countries, the universities are undergoing an unprecedented growth, but Professor Janssen, chairman of the Netherlands Academic Council, said that there are many reasons to reconsider the structure and curricula of the Dutch university system. In particular, the rapidly increasing number of students seeking university places, the long duration of the studies, the high drop-out rate, the manpower demands of society and the rising costs of higher education all demand some change in the university system; but for Professor Janssen, the chief cause for concern is the length of degree courses. "If the Netherlands education system attains the goal of graduates with a shorter period of study," he said, "all these problems would be somewhat alleviated."

The general consensus among Netherlands educational authorities is that a new four-year degree course should be introduced. This would be designed "not to train students for a particular profession but to teach them the scientific method". The Netherlands delegates at the confrontation meeting pointed out, however, that no basic changes in the universities can be made without almost unanimous consent of the universities themselves.

NEW WORLD

Pugwash Worth its Weight in SALT

by our Special Correspondent

Fontana, Wisconsin, September 13

If men should be judged by their deeds, not their words, by the same token the measure of international conferences should not be taken from their press conferences or published statements. This is particularly true of the meetings of the Pugwash movement, whose principal value probably lies in the opportunity afforded for conversations between the American and Soviet participants. Last year's conference, at Sochi on the Black Sea, and this year's, held by the waters of Lake Geneva, Wisconsin, have opportunely preceded the first and third rounds of the Strategic Arms Limitation Talks (SALT) which, if either side regarded them as such, could become the most important political negotiations since those that concluded the Second World War. What has Pugwash to offer to SALT?

In the words of a participant from the eastern bloc of nations, Pugwash cannot remove the stones that may have clogged up the ordinary diplomatic machinery, but it can at least clear away some of the finer grains of sand. Though both sides have undertaken to make no comment on the progress of SALT, the strategic situation alone would afford little hope of the talks producing anything more than an anodyne agreement not to do what neither was intending to do in any case. Yet conversations such as those that occur both publicly and privately at Pugwash may be of inestimable value in suggesting ways round impasses and even simply in educating the Soviet participants in the strategic implications of the present stage of the arms race. If the flow of information and ideas is almost entirely one-sided, that is because the traditional secrecy in which the Soviet Union invests such matters gives Americans a monopoly in figures, statistics, and the ideas on which these are based. Nor is it clear that individual Russians have as firm and comprehensive a grasp of their own side's policy as do their American counterparts who have given technical advice on such matters to the Pentagon and other government agencies. Indeed, it seems that because of the isolation of one government department from another, many Russians may rely rather heavily for their knowledge of Soviet arms and strategy on what they read in the *New York Times* or the yearbooks issued by the London Institute of Strategic Studies and the Stockholm International Peace Research Institute. It may be that from the Soviet point of view the Pugwash conferences would be valuable even if they served as nothing more than a seminar. It can only be a matter of speculation to what extent the private discussions that have taken place this year and at last year's conference have dealt with the specific groundwork of the SALT talks but it would be surprising if these were not touched upon.

For this reason it is hard to understand the view expressed at the conference that now the SALT talks have begun the Pugwash movement has little more to

offer on the subject and should turn its attention elsewhere. The opposite is the case. Disarmament has been the central concern of the movement ever since the first meeting at Pugwash, Nova Scotia, in 1957, and with the start of the SALT talks Pugwash has more to contribute than ever. At this year's session, working groups were formed to discuss the topics of environmental pollution and science in developing countries, but although individual participants may have useful ideas to offer it is hard to see that among a plethora of international organizations Pugwash has a unique contribution to make on these particular issues. Certainly several of the Russian participants made clear that their interest lay in the problems of disarmament and Pugwash without the Russians would be as Hamlet sans prince.

Much of the working group discussion about disarmament was coloured by a Pugwash symposium held two months ago in Racine, Wisconsin, at which it was foreseen that an order of magnitude improvement in the accuracy of long range missiles could be expected within the next five to ten years. This development would mean that the relatively small warheads carried on a multiple independently targetable reentry vehicle (MIRV) could be positioned with an accuracy nearer to 40 than the present 400 metres. This is sufficient to knock out the silo-based missiles of the other side and, were it not for the existence of Polaris-type submarines, would threaten the capability of retaliating after a first strike. If the land based missile is headed for obsolescence, nuclear stability will be maintained by opposing fleets of Polaris submarines. For this reason the SALT negotiators should address themselves to the task of forestalling any development that would threaten the impregnability of submarines.

Already on the horizon is the technology for arrays of powerful sonar devices which, in the absence of appropriate countermeasures, could "make the oceans transparent", or at least reduce their opacity. An agreement not to deploy such devices, and to limit the number of hunter-killer submarines, could be an important consequence of the SALT talks, and certainly of more substance than purely cosmetic instruments such as the undertaking not to nail nuclear weapons to the sea floor.

The progress of the SALT talks was avoided in the working group discussion but there seemed to be few suggestions and little optimism as to how the deployment of MIRV missiles could be avoided. These present a far more serious escalation of the arms race than the anti-ballistic missile systems (ABM) which neither side seems keen on developing beyond the stages already attained, SALT or no SALT. The recent United States announcement that it would proceed to MIRV a few of its Minuteman missiles in December was followed chronologically, and perhaps casually, by the Soviet decision to resume work with its silo-busting SS-9 missiles, deployment of which

had been temporarily halted. The Soviets also profess to regard the timing of the American action as being a deliberate attempt to take MIRVs out of the orbit of the SALT talks.

It is true that the pause in deployment of the SS-9, together with the low level of underground nuclear testing by the Soviet Union (in the first half of this year the USSR conducted one test and the USA 23) could be taken as signs of genuine expectation that the SALT talks might come to something. On the other hand this low activity may have been dictated more by economics than pacific intentions, and it is in any case hard to understand how the Russians, if they take SALT seriously, could have been prepared to jeopardize the mutual trust on which any SALT agreement must be based by their actions during the last few weeks in the Middle East. Even if the Egyptian missiles were only swapped around, the Soviet observance of the truce should, like Caesar's wife, have been above suspicion.

On the American side, too, it is hard to see how the cause of SALT was enhanced by the acceleration of the MIRV programme, and indeed those who see SALT as a superpower diversion to prevent discussions of general and complete disarmament can even argue that but for SALT neither MIRVs nor ABM would have proceeded as far as they have. Although the main strategic implications of MIRVs are as a first strike weapon, they were apparently developed by the United States as a penetration aid against the sophisticated Soviet air defence system which, it is claimed, is capable of being upgraded into an ABM system. In short, there is little outward sign that either superpower holds much hope of SALT. The discussions at Pugwash may serve to persuade the respective governments that the lack of omens is not necessarily a bad omen.

DRUGS

Marihuana and the Law

by our Washington Correspondent

ANOTHER voice has been added to those who are calling for a reconsideration of the laws relating to marihuana. A staff report of the now defunct National Commission on the Causes and Prevention of Violence* recommends that marihuana be made legal for people more than 18 years old. The existence of the anti-marihuana laws, the report says, means that "large segments of our population are engaging in criminal behaviour. Many are caught and labelled 'criminal', despite the fact that they are otherwise law abiding citizens. No other body of legislation has created as much general disrespect for our laws, particularly among the young. No other body of legislation has 'criminalized' as many otherwise law abiding citizens".

The report proceeds to urge drastic changes in the existing "archaic and ineffective" laws, including legalization of the drug for those over 18. The idea of a government licensing system should also be considered so as to pre-empt the market from organized crime.

This verdict is based on a balance sheet with few items. On the one hand the abuse of marihuana can be as dangerous as the abuse of other drugs, such as

alcohol. But the staff report cites a long list of studies including the Wootton report in England (1968), the Mayor La Guardia report (1944) and even the venerable Indian Hemp Drugs Commission (1894) to show that marihuana is not a cause of crime and violence. There is evidence that in the United States, at least, marihuana use does not lead on to heroin or other harder drugs, except in as far as it is the personality of the user, rather than the properties of the drug, that causes progression.

On the other side of the balance sheet is the cost of enforcing the laws against marihuana. The staff report quotes a recent estimate that between 500,000 and 1 million citizens of the United States are regular smokers of marihuana and that between 3 and 5 million use the drug occasionally. Many of the users are young people, and probably include up to a quarter of the students at schools and colleges in metropolitan areas. But marihuana is by no means confined to students; a recent survey of a large American city showed that 11 per cent of the entire population had used the drug.

Even the literal cost of bringing these "criminals" to book is appalling. California alone spent \$75 million on enforcing its marihuana laws in 1968. Arrests for marihuana offences in the state during the first half of 1968 were running at a rate of 31,000 adults and 15,000 juveniles. The consequences of labelling a person as a deviant or delinquent, the staff report points out, are that he often is led to act in conformity with the label. Perhaps the greatest social cost is that "much of our youth has suffered a loss of respect for the law. They note that marihuana use carries serious criminal penalties while the use of alcohol, which evidence shows to be much more harmful when abused, is acceptable and lawful". The report concludes that social costs of the marihuana laws far outweigh the real dangers of the drug.

The sections on marihuana comprise only a small part of the report, which runs to some 1,600 pages and covers all aspects of criminal violence. Much of the material was used by the National Commission, chaired by Dr Milton S. Eisenhower, in its Report to the President. The Commission did not, however, adopt the recommendation on legalizing marihuana but merely called for more study of the question. Printing of the staff report has been delayed until now because of lack of funds.

There is at present no chance at all that the report's recommendation concerning marihuana will be acted on. The Attorney General, Mr John Mitchell, for one, remains persuaded that it is a dangerous drug. There are also serious political risks that face the party or state legislature seeking to take a softer line on marihuana. Nevertheless, the times are changing. A few days before the staff report was published an issue of *Newsweek* asked on its front cover whether it was time to change the law. Even Senator Barry Goldwater has publicly enquired whether the existing laws are perfect. It seems there is also a tendency away from allowing special interest groups to inflict their own moralities on the community. The Roman Catholics and the medical associations, for example, have been required to yield on abortion, just as in the 1930s the Methodists and others were forced to give way when Prohibition proved unworkable. For that matter the laws against marihuana have become so widely abused as to appear to many as a new Prohibition.

* *Crimes of Violence*. Pp 1,597. US Government Printing Office, \$6.50.

EARTHQUAKES

Miracles and Myths in Alaska

by our Washington Correspondent

THE great earthquake that struck Alaska late in the afternoon of Good Friday, March 27, 1964, was far less terrible in its consequences than would be expected for an event registering between 8.3 and 8.75 on the Richter scale. Only 115 lives were lost in Alaska and the tsunamis engendered by the quake took only a handful elsewhere. And just as the earthquake at Lisbon inspired Voltaire to write *Candide*, the Alaskan earthquake has moved the National Academy of Sciences, if not to such heights of philosophy, at least to the ambition of making the event the best documented earthquake in history.

Less than a day and a quarter after the earthquake had struck, a team of five sociologists from the Disaster Research Center at Ohio State University descended upon the area and began to interview the actors in the tragedy. This and much other material has been combined in a description of how people coped with the earthquake, both in the immediate aftermath of the disaster and in the following days when the initial shock had worn off. The document* is of considerable interest in showing the great resilience of human societies in the face of a major, though far from crippling, disaster.

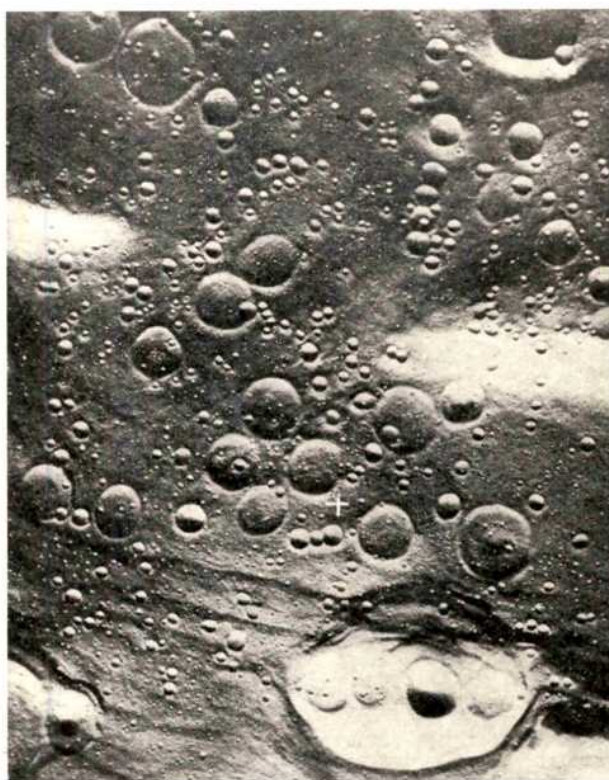
The Alaskans attributed their energetic behaviour during the calamity to their frontier spirit: "This is a frontier country and we are used to hardship" was the verdict of radio announcers, editorial writers, and people in bars. The Ohio sociologists bluntly state that the frontier spirit is a myth. The Alaskans behaved courageously to be sure, but no better than the average behaviour in the face of disaster. In Alaska, as elsewhere, the heady heroism and altruistic gestures later gave way to private interest after the worst danger had passed. Firms that had given free supplies or equipment began to expect reimbursement and individuals demanded payment for long hours of overtime work.

The Ohio Disaster team also comments on the tendency for people to do what they are trained to do in the hour of crisis, not what most needs doing. Policemen look for traffic to direct, firemen seek fires to fight, utility workers restore utilities and administrators hold meetings, but people are disinclined to take on novel tasks such as organizing rescue parties to search the debris. Systematic search and rescue efforts did not begin until more than 12 hours after the earthquake. In Anchorage, at least, people seemed to give a greater priority to protecting property than saving lives, in the mistaken belief that there would be heavy looting. The need to assert control over the physical surroundings seemed more compelling to most people than that of digging among the rubble for survivors. Officers of the Salvation Army, for example, spent the first half hour after the earthquake helping police to direct traffic. The police department's first thought was to place security guards at intersections and buildings.

The first reaction of the Eskimo villagers was perhaps more apposite than that of the townsfolk in Anchorage. Within ten minutes after the shock the

people of Tatitlek in Prince William Sound were holding a service in their church while the walls were still shaking. As an act of God the earthquake was not wholly inopportune since for several months afterwards it revitalized attendances at the Russian Orthodox church, the prevalent religion among Alaskan Eskimos. The tsunami created by the earthquake made a particularly dramatic intervention at the village of Old Harbor on Kodiak Island, where it washed away the rival Protestant chapel but spared the Russian Orthodox church. The church's situation on higher ground did not prevent the sect's adherents from ascribing its survival to a miracle. A year previously, a small earthquake had occurred on the same night as a lay reader abandoned the orthodox church for the protestants and a whole family was about to apostasize on the day the Good Friday earthquake struck. It was little wonder that in the face of such celestial odds the protestant missionary left the village soon afterwards.

If the Eskimo villagers learnt the message of the earthquake, that was more than could be said for the businessmen in Anchorage. In 1960 the US Geological Survey had warned that much of Anchorage was built over a layer of unstable clay. These areas, some of which subsided as much as 70 feet in the

Target for Apollo 14

The cross marks the landing site in the Fra Mauro formation for the Apollo 14 astronauts, Commander Alan B. Shepard, Command Module Pilot Stuart A. Roosa, and Lunar Module Pilot Edgar D. Mitchell. NASA now says the launching will be no earlier than January 31 next year. Fra Mauro is a flattish highland area roughly 110 miles nearer the centre point of the Moon's near face than the Apollo 12 landing site in the Ocean of Storms.

* *The Great Alaska Earthquake of 1964: Human Ecology*. National Academy of Sciences, Washington DC. \$29.50.

quake, were declared high risk areas and ineligible for government reconstruction aid. But relocation of the business area was said to be too expensive by the businessmen and little by little concessions were made. Now, six years after the earthquake, new buildings have been constructed on top of the very area that suffered sliding, and an apartment house and a luxury hotel have been built in a designated high risk area next to the slide.

Changes at MIT

THE presidency of the Massachusetts Institute of Technology will fall vacant in June next year when Mr Howard W. Johnson steps down to become chairman of MIT's Board of Trustees, known as the corporation. Mr Johnson will take the place of Mr James R. Killian, former science adviser to President Eisenhower, who has announced he will retire then.

Mr Johnson has been president of MIT since July 1966 and will have served a full five years in office which, he says, "have been the best five years of my life". But he has made no secret of the arduous nature of the job and has recommended that the Board of Trustees should reassess the presidency to see how some of the responsibilities might be shared among colleagues.

Another change at MIT is the appointment of Dr Eugene B. Skolnikoff as head of the Department of Political Science. Dr Skolnikoff served as assistant to Dr Killian, Dr George B. Kistiakowsky and Dr Jerome B. Wiesner during their terms as science adviser to the president.

METEOROLOGY

Microscope on St Louis

from a Correspondent

A LARGE-SCALE cooperative study of the impact of a large city on the atmospheric environment is to be launched in 1971. A planning meeting bringing together the various scientists concerned recently took place at the National Center for Atmospheric Research (NCAR), Boulder, Colorado. The object is to study the sources and effect of urban pollution and the physical and chemical reactions produced by pollutants in the atmosphere. St Louis has been chosen as the target city because it is neither a grossly polluted area nor an exceptionally clean one, so that conclusions would apply widely elsewhere. This will be a multiple research project with six groups taking part, though not necessarily at the same time, and will take several years to complete.

The first part of the programme will test the theory that urban air pollution affects rainfall patterns downwind from a large city. This is to be handled by a team drawn from the Illinois State Water Survey, the University of Chicago, the University of Wyoming and the Argonne National Laboratory. It will take five years, and will employ 200 rain gauges, three radar systems, several standard weather stations and specially

instrumented aircraft. The Environmental Science Services Administration (ESSA) has proposed work on mesoscale weather prediction involving computerized numerical forecasting of medium-scale features such as thunderstorms using a dense, three-dimensional network of weather stations.

NCAR itself plans the systematic monitoring of persistent pollutants well downwind of the city using ground stations and aircraft. It is hoped that this will be a first step in predicting which pollutants (if any) have lifetimes long enough to threaten contamination of the atmosphere on a continental or even global scale. There has been much alarm but little evidence of this possibility in the United States; there has been less of both elsewhere—indeed, the latest report of Britain's alkali inspectorate publicly pooh-poohs the idea (*Nature*, 227, 875; 1970) as speculative. It will be useful to have some facts on the matter at last. Previous American observations (quoted in the recent Presidential report on environmental quality) have shown that large American cities have more rain, more cloud and more fog than the surrounding countryside, and that there is a "thermal mountain" in the atmosphere above them.

CANADA

Doing More with Less

Two chief themes run through the report of the National Research Council of Canada for the financial year 1969-70. The year was dominated by financial stringency which seems to have caused much soul-searching and reassessment of priorities—especially in the NRC university support programme—and the council was clearly concerned to improve its sponsorship of applied research. The National Research Council, a federal government agency charged with the task of supporting both university and industrial research, last year spent \$132.7 million; \$65 million went on university research grants and scholarships, while \$6.3 million found its way to research in industry.

One of the NRC's responsibilities is to look after the external relations of Canadian science, and it must therefore have been a painful decision to abolish travel grants for Canadian university scientists when the council was forced to take in its belt. But the council is clearly looking towards greater rationalization and concentration of its resources—in much the same way as the British Science Research Council has done—to gain the maximum benefit from its limited funds. Unfortunately, however, the machinery for making choices between various projects is only now evolving in the NRC, and it is looking towards the Science Council of Canada—the chief policy making and advisory body—to give some lead.

Stung, perhaps, by the remarks of the OECD enquiry into Canadian science policy earlier this year (see *Nature*, 224, 1054; 1969), the NRC has been taking a good look at its activities in the industrial research field. A small group of scientists, engineers and economists has been assembled to draw up long range plans and to review existing policies, and its chief tasks will be to improve the relationships between the universities and industry, while at the same time keeping a wary eye on the likely demand for scientific manpower.

NEWS AND VIEWS

Laugh Kindly at the Policy Sciences

EVEN in the higher reaches of intellectual life, there is a tendency to think that giving a dog a good name is tantamount to sanctifying him. One of the most recent and most spectacular illustrations of this principle is the eagerness with which the name "policy sciences" is catching on among the well intentioned body of men now seeking to apply quantitative methods to a variety of problems previously considered in terms which are too vague. The trouble, of course, is that the term does not refer to a definable area of intellectual investigation but rather to what may be a method, an attitude of mind or even a hope about the way in which diverse problems should be tackled. In the circumstances, it is perhaps understandable that writings in the policy sciences should so often run to jargon and incoherence but it is, of course, less easy to understand why intelligent people with reputations to lose should lend themselves as willingly as they seem to have done to this misguided cause.

The epitome of all this is a new journal *Policy Sciences* which appeared earlier this year (American Elsevier, New York, \$15.00 per annum). The editor, a Dr E. S. Quade of the Rand Corporation, sets out to explain what the policy sciences are about. He says that in the past thirty years there has been "almost a revolution . . . in basic thinking about the nature of policy and how it is or should be made". What he has in mind is the application of operational research, systems analysis, gaming and the like to problems in military strategy and in management. So why not attempt to introduce the same methods for the solution of problems usually tackled by social scientists, political scientists and officials responsible for public administration? With this moderate demand, few will disagree. The question unanswered by this modest declaration is whether the policy scientists would best achieve their aims by working within the existing fabric of intellectual life or separately, under a banner of their own. *A priori* answers to questions like these are, of course, untenable but unfortunately there is nothing in the first issue of the policy scientists' new journal to suggest that what they have to say is distinctive enough and fresh enough to justify a separate existence.

Failure may nevertheless be illustrative of deeper truths. The new journal contains two articles which presumably typify the kinds of problems with which the policy scientists think they may be able to help. Thus Dr Stephen Enke has an interesting calculation of the prospective consumption of goods and services by an infant growing to maturity and a balancing calculation to show how much the same individual will produce during his lifetime. Not surprisingly, the two

figures are more or less in balance, but this kind of calculation is a necessary starting point for the demonstration that nations in which there is a rapid growth of population are less able to sustain a rapid growth of prosperity as well. The trouble, of course, is that there is nothing in the policy sciences to suggest which of the several incentives which might be introduced as means of regulating the growth of a population is likely to be most effective. So, at the end, the policy scientists find themselves appealing to the social scientists. The outcome of Dr Vincent Taylor's attempt to calculate the value of good health is much the same—here too the chief conclusion is that health services should be more responsive to the demands that ordinary people would make on them if an awareness of what health services could provide were more generally available.

This, however, is modest stuff. The extravagances of the policy sciences are more clearly to be seen in the contribution by Dr Yehezkel Dror which spells out the way in which the "establishment of policy sciences as a new supradiscipline involves a scientific revolution requiring fargoing innovations in basic paradigms". What Dr Dror seems to mean by this is that there should be a striving after the worthy but nebulous goals of breaking down interdisciplinary barriers and those between pure and applied research, the bridging of the gap between the past and future and the exploitation of "tacit knowledge" in the formulation of decisions. In his article, Dr Dror makes quite clear his belief that the coming of the policy sciences will work important changes in the fabric of society. He considers that "the whole nature of politics may well change . . . with some policy science units being constitutionally charged to present their analyses and recommendations before both the elected bodies and the public at large". Similarly, he considers that there will have to be changes in the ways in which "prime television time" is allocated so that the policy scientists can have their say about controversial issues when people are most likely to be glued to their television sets. In the same transformation of society, steps would be taken to educate politicians, there would be a systematic evaluation of past policies and radical changes in school curricula. In no time at all, Dr Dror's prescription for the future turns out to be substantially the same as a great many well intentioned but platitudinous hopes for the future. Of course, it would be valuable if the political process were more rationally conducted and if governments were better placed to evaluate the consequences of past acts and to anticipate the consequences of future actions. But there is no serious reason to think that

what Dr Dror and his colleagues call the policy sciences are likely to contribute more to the improvement of political governments than, say, political movements themselves. Indeed, if the policy scientists talk too much as Dr Dror has written, there is a serious danger that they will be thought to be as overweening in their pride of knowing how to put society right as were the physicists only a short time ago. In short, they seem well on the way to spoiling a good idea with too much ambition, too little thought and too much bad language (in the sense of jargon).

PALAEOMAGNETISM

Permanent Non-dipole Fields

from our Geomagnetism Correspondent

THERE has been a quiet revolution in geomagnetism during the past few years. Spherical harmonic analysis, for example, which has dominated geomagnetic thinking for more than a century and is still far from redundant, has begun to give way to physically more meaningful models which are, in principle if not in detail, strangely akin to early theories for the origin of the field. Ideas on the variation of the field are changing too. The oversimplified view of a westward-drifting non-dipolar field is coming to terms with the fact that some components do not seem to drift at all and that in the past the drift may even have been eastwards. The theory of the standing as well as drifting field, developed by Professor Yukutake of Japan in recent years, is an attempt to reinterpret some of the finer details of field behaviour.

Most of these ideas have inevitably been concerned with the Earth's field as directly observed, simply because direct measurement gives the most detailed picture of the real field. But Wilson (*Geophys. J.*, **19**, 417; 1970) has made an important contribution to the new ideas by extending their observational basis backwards by means of palaeomagnetic data. Lest it be thought that palaeomagnetic data are, *a priori*, too crude for this type of work, it should perhaps be said that Wilson has selected only the best results according to predetermined criteria. Chief among these are that each mean palaeomagnetic pole position should be derived from at least twenty oriented rock samples and that the error (α_{95}) of the final mean pole should be no greater than 12° . The analysis has also been limited to the Holocene, Quaternary and Upper Tertiary. These criteria are not, of course, particularly stringent but they nevertheless exclude a high proportion of palaeomagnetic data.

Several interesting features of the ancient geomagnetic field emerge from Wilson's analysis of the eighty-three "acceptable" pole positions. The first is that, although the overall mean pole coincides almost exactly with the geographic pole, the individual mean poles from each source area fall systematically on the side of the geographic pole far from the source. The persistence of this phenomenon over several million years naturally casts doubt on the validity of the geocentric axial dipole hypothesis which is the basis of palaeomagnetic interpretation. The fact that the geographic and grand mean palaeomagnetic poles coincide confirms that since at least the Upper Tertiary the Earth's main field has been axially symmetric.

But the other evidence suggests that it has not been exactly centred.

This deduction is supported by the second feature to emerge from the data (now including those from sea cores), which is that the overall palaeomagnetic inclination is shallower in the northern hemisphere, and steeper in the southern hemisphere, than would be expected from a geocentric axial dipole. If the main field really has been geocentric, axial and dipolar over the period considered, these results would imply a northward component of crustal movement which would have amounted to about 400 km during the past two million years. This would have produced obvious geological disturbances which are not in fact observed.

Wilson's analysis can only mean that since the Upper Tertiary the geomagnetic dipole has not been quite centred. One can then devise any number of models to accommodate the non-central dipole, but the simplest one to account satisfactorily for all the data is to displace the central dipole northwards along the rotational axis. Wilson shows that the displacement derived from palaeomagnetic data on rocks less than two million years old is 191 ± 38 km. The displaced dipole model is, moreover, amenable to multipole analysis, which shows that the off-centre dipole is equivalent to a centred dipole together with a centred quadrupole. The non-central dipole thus contains "the essence of a non-dipole field component"; and it is tempting to equate this with Yukutake's standing non-dipole field.

Does this model, then, invalidate the geocentric axial dipole hypothesis which is essential to palaeomagnetism? Clearly it does; but it should be noted that the dipole displacement is small enough to make it only a second order effect. Angular deviations in low and middle latitudes, for example, are only a few degrees. Nevertheless, as Wilson points out, even this is sufficient to put paid to one use of palaeomagnetic data—testing for Earth expansion. Non-central dipole deviations will easily obscure the very small effect of an expanding Earth on palaeomagnetic directions.

HIGH ENERGY PHYSICS

New Tracks for Fast Particles

SPURRED on by the drive towards accelerators of ever higher energy, A. I. Alikhanian *et al.* of the Yerevan Physical Institute in the Soviet Union have proposed a new method for measuring the energies of very high energy particles by detecting the emission of X-rays in a streamer spark chamber (*Phys. Rev. Lett.*, **25**, 635; 1970). Although the possibility of extracting information on particle energies from transition radiation has been recognized for many years, it is only since the completion of the 70 GeV accelerator at Serpukhov about two years ago that interest has focused on exploiting this technique.

The usual method of measuring energies by Cerenkov radiation becomes impractical at ultrarelativistic energies. It becomes almost impossible to distinguish between particles with the same momentum and different velocities at these energies, and the problem of sorting out K mesons from π mesons, for example, becomes a real headache. The advantage of measuring transition radiation, on the other hand, is that the

intensity of radiation increases linearly with energy in the direction of particle motion, and becomes quite high at relativistic energies.

Transition radiation is produced when a beam of charged particles strikes a discontinuity characterized by a sharp change in refractive index. This might be the boundary of a solid, for example, or as Alikhanian *et al.* have found, even the irregularities of a piece of foam. L. C. Yuan, C. L. Wang and S. Prunster (*Phys. Rev. Lett.*, **23**, 496; 1969) carried out experiments with positrons and found that sufficient X-ray transition radiation was produced to indicate the potential of this method of detection at very high energies.

Alikhanian *et al.* have now shown how the incorporation of a streamer spark chamber to analyse the radiation can add substance to these ideas. They demonstrated the method with a beam of electrons at energies between 1 and 4 GeV. Transition photons were produced by passing the beam through a layered target and the photons and incident electrons were fed into a streamer spark chamber containing helium, neon, iodine and xenon. The photons generated photoelectrons in the spark chamber which were then identified from the ensuing tracks. The principle of the streamer spark chamber is to trigger a high voltage for a time short enough to prevent sparks forming and yet sufficiently long enough to allow the formation of streamers.

Alikhanian *et al.* point out that a real advantage of this method is that the same device detects separately both the radiation and the particles. They say that the inclusion of xenon in the chamber makes it very efficient for detecting photons and they found that the intensity of radiation did indeed go up linearly with the electron energy. They also stress that replacing the layered target by plastic foam did not impede the experiment, and that this augured well for the design of high energy detectors.

They express high hopes for the system in the coming generation of accelerators of several hundred GeVs and claim that the measurement of energy by this means, when the particle momenta are defined by placing the spark chamber in a magnetic field, affords a valuable way of identifying particles in the very high energy arena.

UPPER TEESDALE

Dedication on Cow Green

by our Botany Correspondent

SINCE 1967 botanists have been busy in Upper Teesdale, spending the £100,000 given by ICI for research in and around the area of Cow Green that is soon to become a reservoir. Although some of them have seemed a little reluctant to reveal what they are up to, the local contingent gave a progress report on September 7 to interested members of the British Association in Durham. Apart from the excitement of snatching plants from the site of the reservoir before the water pours in, there has been a great deal of dedicated ecology in the wind and rain which are common in that part of the world.

The ancient question of whether the rare arctic-alpine plants of Upper Teesdale could have survived

there for more than 10,000 years in spite of the improving British climate has been tackled by Dr Judith Turner (University of Durham) and her team of pollen analysers. They have found that rarities, including *Gentiana verna*, *Dryas octopetala* and *Betula nana*, provided 15-40 per cent of all the pollen preserved since the retreat of the ice. Dr Turner feels that the eleven rarities she has been able to identify by their pollen are sufficient to show that conditions were always suitable for the success of the seventy-five rarities now recognized in the area. Furthermore, pollen counts indicate that even when forests were densest in Upper Teesdale, there was no closed canopy on the fells, which could have prevented the survival of the small arctic-alpine plants.

Also on Cronkley Fell, Dr A. W. Davison and Mr T. J. Bines (University of Newcastle upon Tyne) are investigating the pressures which seem to be threatening the survival of rarities on a small area of grassland known as Thistle Green. Their intention is to produce a management plan for the conservation of the area, which is beset by wind erosion, grazing sheep and burrowing rabbits and moles. The building of an enclosure to exclude the sheep from a trial area proved to be a costly operation, involving winches to haul posts to the top of the fell. As expected, the immediate result of this exercise was a more lush growth of some species. Although sheep seem to be causing damage by overgrazing, moles are less of a threat. Apparently in this area plants are killed when they are buried under molehills, but there are too few moles to do great harm to any rarities.

Dr David Jeffrey (Trinity College, Dublin) has a more specific project; he wants to know why *Kobresia simpliciuscula* has survived in Britain only in Upper Teesdale, above 1,500 feet, and in the highlands of Scotland, above 2,500 feet. After applying phosphate and nitrate to the soil he has concluded that, in Upper Teesdale at any rate, the growth of grasses which would compete with *Kobresia* is limited by the availability of phosphate. Although there does not seem to be a shortage of phosphate where *Kobresia* grows, the presence of lead as galena (PS) seems to depress the ability of grasses to utilize phosphate. Thus they are effectively deprived of certain nutrient material and so do not grow at full strength, leaving *Kobresia* free to flourish.

Dr M. E. Bradshaw (University of Durham) and her followers have several projects in progress on Widdybank Fell. So far fifty-five acres have been searched by amateur ecologists who are helping to map the distribution of the vegetation. Work is also in progress on the population dynamics of selected species at various distances from the water line of the reservoir. It is hoped that the effects on the populations of a large body of water will gradually become apparent.

Two projects which have almost reached their deadline involve the collection of plants from the area to be inundated. About 3,000 herbarium sheets have been produced in an ambitious scheme for the preparation of thirty sets of all the flowering plants on this site. These will be distributed to herbaria around the world. Living plants have also been scooped up, and have mostly gone to Durham and Manchester, where they are to be studied in captivity. At Durham they are thriving in large pots made from lengths of sewage pipe. Among these species is *Carex paupercula*,

the only plant that will be lost completely from the part of Teesdale which is to be flooded.

As well as flowering plants, algae have been taken to Durham, and a stream is being built where Drs B. A. Whitton and M. K. Hughes hope to be able to study communities taken from calcareous flushes. The prominence of the blue-green alga *Rivularia basiolettiana* makes these communities unique in such cold water.

SKIN CANCER

DNA Repair Defect in Rare Disease

from our Medical Biochemistry Correspondent

ALTHOUGH mutagens are known to be implicated in human cancers, there is little direct evidence of alterations in DNA or DNA metabolism. Some recent work which suggests that there is an inherited deficiency in the DNA repair system in the disease xeroderma pigmentosum is therefore of great interest. This disease is a rare inherited condition in which the skin is extremely sensitive to ultraviolet light and numerous skin cancers develop. In the more severe forms of the disease, called the de Santis-Cacchione syndrome, there are neurological disorders as well.

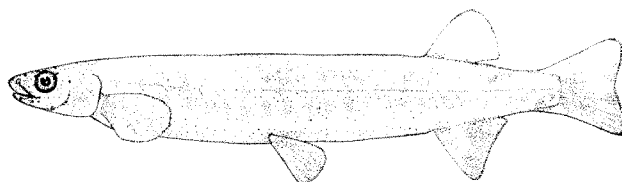
It has previously been shown that tissue cultures of fibroblasts from patients with this disease do not incorporate tritiated thymidine into DNA after ultraviolet irradiation as well as do normal fibroblasts. This work suggested that there is an inherited defect in the repair system of unspecific DNA synthesis, and now Epstein *et al.* (*Science*, **168**, 1477; 1970) have shown that this effect can be observed *in vivo* and that the defect affects all types of cells in the skin. They gave eight normal controls and three patients with xeroderma pigmentosum irradiations of 13.6×10^6 ergs/cm² of ultraviolet light at wavelengths of less than 320 nm. Tritiated thymidine was injected 15 min later into the irradiated area and adjacent unirradiated skin and, after 1 h, biopsies were taken for sectioning and autoradiography. There was dense labelling indicating DNA synthesis before cell division in 5 per cent of the basal cell nuclei and in 1 per cent of the dermal fibrocytes. Sparse labelling of three to fifteen grains per cell occurred in a very few unirradiated normal cells but, in the irradiated normal samples, about 500 out of every 1,000 cells of all types had sparse labelling. Two of the patients had the severe form of xeroderma pigmentosum with neurological abnormalities and these showed no sparse labelling at all after irradiation. The other patient had skin changes only and, after irradiation, his cells showed some sparse labelling ranging from 4 per cent of that in the normals in the upper dermal fibrocytes to 20 per cent of the normal value in the basal cell layer. There is therefore clear evidence that patients with this inherited defect are unable to respond to irradiation by DNA repair in the normal way, and the enzymological change responsible for this should be interesting. This defect is very probably the cause of their skin cancers, and because ultraviolet irradiation seems to cause skin cancer in normal humans, the inefficiency of the DNA repair system might well be a factor concerned in the development of other skin cancers.

GALAXIID FISHES

Speciation in New Zealand

from our Marine Vertebrate Correspondent

NEW ZEALAND has been geographically isolated from other land since at least the end of the Mesozoic and its native freshwater fish fauna shows this long isolation by its relative poverty. Only thirty-one species are known, compared with 127 in Japan and seventy-two in the British Isles, and fourteen of these species belong to the family Galaxiidae. This suggests that this family may be the oldest existing element in the freshwater fish fauna and, as such, it is of considerable interest. In addition, the study of this family is likely to shed light on speciation within the group in New Zealand and their relations with other galaxiid fishes elsewhere. A recent publication by R. M. McDowall (*Bull. Mus. Comp. Zool. Harv.*, **139**, 341; 1970) marks a major advance in knowledge of these fishes.



Galaxias maculatus.

Most of the galaxiids are small and scaleless, with rounded trunks and rather broad heads. They tend to be solitary and are usually rather secretive; some live in overgrown pools and are nocturnal in habit, while others have midwater shoaling habits. Body shapes and fin form tend to respond to these varying habits. One of the most extreme New Zealand habitats is that in which the fishes of the genus *Neochanna* live. McDowall reports on a kauri-gum swamp at Waiharara, South Island, in which there were many small depressions a few inches to several feet across and up to eighteen inches deep. These holes were heavily overgrown with sphagnum and filled with twigs to the extent that they were scarcely distinguishable from the surrounding swamp. This unpromising area proved to be the major habitat of the species *Neochanna diversus*. It seems very likely that this and the related species aestivate during the dry season, when their ephemeral habitats dry up, or at least become deficient in oxygen.

By contrast with this species, other New Zealand galaxiids are diadromous, the juveniles being well known as "whitebait" during a shoaling, migratory phase when they enter the estuaries on their return to the rivers in which they live. *Galaxias maculatus* is the most commonly encountered of this type, and is widely distributed both in New Zealand and elsewhere. This species is peculiar in that it breeds amongst grasses on estuarine flats synchronously with the spring tides. The eggs are deposited amongst the bases of grasses and sedges and do not hatch until the next cycle of spring tides, when the larvae are carried out to sea. The young fish are found as migratory whitebait in the estuaries in the subsequent spring some six months after spawning. McDowall confirms that this species is highly fecund, and its eggs are very small when compared with the freshwater

species which lay relatively few, large-yolked eggs, which is not unexpected in view of the high mortalities normal in the marine plankton.

Galaxias maculatus is also seen to be the most widely distributed, being found in southern Australia, and the southern tip of South America, as well as New Zealand. This in itself is not surprising in view of its spawning habits which would assist dispersal by ocean currents. From this and the evidence of the distribution of the other galaxiid species in Australia and New Zealand, McDowall suggests that they evolved in the Australasian region, and that their establishment elsewhere has been by trans-oceanic dispersal. Confirmation of this may depend on systematic studies of the Australian and South American galaxiid faunas both of which are very imperfectly known.

MYOSIN

What the Early Burst Catches

from our Molecular Biology Correspondent

It can be argued that any attempt to define the nature of the myosin-actin interaction, which lies at the heart of the mechanism of muscular contraction, would be otiose without a prior analysis of the myosin ATPase reaction. Two papers from Taylor's laboratory in Chicago go a long way towards dispelling the fog into which a mass of scattered and undigested data had plunged the enzymology of myosin. The key to the ATPase mechanism lies in the old observation that the course of steady-state hydrolysis extrapolates at zero time to a finite value for the number of protons released. This is the so-called "early burst" phenomenon, and its origin has been a matter of conjecture. Lymn and Taylor (*Biochemistry*, **9**, 2975; 1970) have now studied the kinetics of the early burst, which occurs within a time-scale of the order of 0.1 s. Their method consists in arresting the reaction at chosen times by rapid introduction of acid, and then determining the total phosphate released from labelled ATP within the total reaction time. The kinetics, especially at relatively low ATP concentrations, adhere closely to a single-term rate equation. The magnitude of the total burst is a function of ATP concentration, and extrapolates to a maximum of 1.8 moles of phosphate released per mole of myosin—that is to say essentially one for each active site. One of the most striking features of the reaction is that the early burst occurs, and with the same kinetic characteristics, both in the presence of calcium and of magnesium, even though the latter strongly inhibits the ATPase, as normally measured in the steady state. In the presence of EDTA, which activates the ATPase, the early burst is completely eliminated, and instead a lag phase of some 0.1 s precedes the attainment of the steady-state rate. Now this pattern is characteristic of the simplest model for an enzymic reaction, whereby an enzyme-substrate complex is first formed in a concentration governed by a single binding constant, and then decomposes to enzyme and products by a first-order process. The lag is a consequence of the accumulation of complex, and the Michaelis constant can be derived from measurements of the lag as a function of substrate concentration. The value thus obtained for myosin agrees with that derived in conventional manner from steady-state velocities.

With calcium or magnesium, on the other hand, the relation between rate in the transient phase and the size of the burst is entirely consistent with the presence of a second reaction intermediate, in the form of an enzyme-product complex. The lag phase is not here observed because of the rapidity with which the enzyme-substrate complex is converted to the enzyme-product complex. Once the latter has been formed, with release of a proton for each active site, it is its dissociation that becomes rate-limiting. It is at this point in the scheme that the behaviour of calcium and magnesium ions differs, and at which also actin must be presumed to mediate the reaction.

In the accompanying paper, Taylor, Lymn and Moll (*ibid.*, 2984) enlarge on the properties of the enzyme-product complex. Their experiments are based on the exclusion of myosin, and not of ATP or free products from the matrix of a gel-filtration column. A zone of ATP is run into the top of the column and a band of myosin is then rapidly passed through. The extent to which the two reaction products, ^{32}P -labelled phosphate and tritiated ADP, cleave to the myosin can be determined as a function of time by comparing the radioactivity elution profiles with that of the protein. Under standard conditions, in the presence of magnesium, it turns out that both ADP and phosphate are bound, and their rate of dissociation from the myosin is in gratifying agreement with the steady-state rate of magnesium-inhibited ATP hydrolysis. In the presence of calcium, the dissociation rate is, as expected, too fast to measure. These critical experiments leave little doubt that it is indeed the dissociation of the products, in particular the ADP, that governs the hydrolysis rate, and this simple mechanism makes redundant earlier and more complex schemes. There is some evidence from non-linearity of binding plots which raises again the possibility that the ATPase sites on the two myosin heads may not be completely independent.

An investigation of myosin by attachment of a spin-label to the thiol groups comes from Saidel, Chopek and Gergely (*ibid.*, 3265). The broadening of the ESR signals from the label shows that its mobility becomes severely restricted on binding. Addition of ATP or pyrophosphate leads to an increase in the mobility, the effect becoming maximal at a ratio of two moles pyrophosphate per mole of myosin. The label must be supposed to respond to some small localized structural change. It is interesting that the effect of ATP persists even after inactivation of ATPase activity by chemical modification.

VIRUSES

Going it Alone

from our Cell Biology Correspondent

THE twin concepts of defective viruses and helper viruses have played an important part in the development of RNA tumour virus biology. Increasingly, however, it is becoming obvious that the supposed defectiveness of many RNA tumour viruses is an artefact of the way they are studied and ignorance of the factors which determine the host range of particular viruses. For example, Bryan high titre Rous sarcoma virus was for many years described as defective because its envelope had to be supplied by an avian

leukaemia virus. But three years ago Vogt, and then Hanafusa, found that this sarcoma genome replicates and produces infectious progeny, envelope and all, without the help of a leukaemia virus when it infects quail cells and cells of certain strains of chicks. The RSV.O genome, as it is designated, is not defective in all cells. Now Aaronson, Jainchill and Todaro (*Proc. US Nat. Acad. Sci.*, **66**, 1236; 1970) report that murine sarcoma virus, contrary to popular misconception, can transform mouse cells without the help of a murine leukaemia virus.

When mouse cells are infected with serial dilutions of stocks of murine sarcoma virus, which always contain murine leukaemia virus, the number of foci of transformed cells decreases as the square of the dilution. This classic observation led Rowe and Hartley (1966) to conclude that transformation by murine sarcoma virus depends on the cell also being infected with murine leukaemia virus as helper. Such transformed cells yield progeny sarcoma viruses, sarcoma genomes in leukaemia virus envelopes and progeny leukaemia virus.

Recently, however, Aaronson and Rowe isolated a transformed mouse cell which yielded neither sarcoma nor leukaemia virus, but when it is superinfected with leukaemia virus alone the progeny contains sarcoma virus as well as leukaemia virus. This suggested that this cell had been transformed by a murine sarcoma genome in the absence of a leukaemia genome. Aaronson *et al.* have confirmed this interpretation. When mouse cells of the BALB/3T3 line are infected with serial dilutions of murine sarcoma virus, the number of foci of transformed cells, counted 3 days after infection, decrease, as the square of the dilution; this is the classic result. But when the same culture is counted 7 days after infection there are more foci and their number is proportional to the dilution, not the dilution squared.

This means that transformation is really a one hit process. The murine sarcoma genome alone can transform but the transformed cells for some reason grow slowly. Those cells which are infected with sarcoma and leukaemia genomes, on the other hand, produce foci quicker because they yield progeny virus of both types which can infect and transform adjacent cells. Claims that the murine sarcoma genome cannot, unhelped, transform mouse cells are an artefact of the experimenter's haste to count the foci in his Petri dishes.

Although the murine sarcoma genome is not defective for transformation it cannot as far as is known specify an infectious virion envelope; only cells infected with leukaemia and sarcoma genomes liberate progeny sarcoma virus. In 1967 the same was being said of the RSV.O genome, but then this virus was found to replicate unhelped in quail cells and some chick cells. How long will it be before a cell which supports the productive infection of murine sarcoma virus is discovered and the last vestiges of defectiveness are forgotten?

NUCLEAR MAGNETIC RESONANCE

Vital Biological Tool

from a Correspondent

It would be an overstatement to describe "retrenchment" as the keynote of the conference on magnetic resonance in biological systems, a NATO Advanced Study Institute, held in Oxford from August 26 to

September 2. But there seemed to be greater emphasis on theoretically sound interpretation of complex nuclear and electron magnetic spectroscopy than before.

When membranes were the topic for discussion, spin labelling received most attention. The cholesterol spin label analogue, cholestane, has been used extensively as a probe in multibilayers of phospholipids, and Dr I. C. P. Smith (National Research Council, Ottawa) described the rotational freedom of the probe on apparent ordering of multibilayers on addition of cholesterol and disruption with solvents and antibiotics. The attachment of a spin label to substituted fluorophosphates provided a convenient reagent for Dr L. H. Piette (Hawaii) to attempt specific labelling of synaptosome acetylcholinesterase.

Electron paramagnetic and nuclear magnetic resonance studies of haem proteins seem to be progressing along two overlapping lines. First, there are investigations of the observed spectra in different proteins, and second, specific studies of haemoglobin aimed at the elucidation of the mechanism of cooperative oxygenation. Drs R. G. Shulman (Bell Telephone Laboratories, Murray Hill) and M. Karplus (Harvard University) presented a formidable resolution of the nature of the high and low field proton magnetic resonances in myoglobin and haemoglobin. They managed to calculate the relative contributions of the contact and pseudocontact shifts in paramagnetic forms.

Conformational analysis, isotope substitution and histidine C-2 proton titrations were in favour with the protein resonators. Paramagnetic probe ions, especially manganese, have been used extensively, both as a phenomenological spectroscopic parameter to investigate binding sites, and, in favourable cases, to determine the stereochemistry of the binding mechanism. Dr M. Cohn (University of Pennsylvania) reviewed this method of investigating enzyme-substrate interactions, and Dr A. S. Mildvan (Institute for Cancer Research, Philadelphia) presented a thorough study of the enolization and elimination mechanisms in pyruvate carboxylase and aldolase. A detailed study of the spin-lattice relaxation rate of water in manganese-carboxypeptidase solutions presented by S. H. Koenig (IBM Watson Laboratory, Columbia) covered the frequency dependence over the 10 kHz to 100 MHz range, illustrating, in its complexity, the difficulties of interpreting temperature dependence of relaxation times at one or only a few field strengths. G. Navon and A. Lamir (Tel-Aviv) illustrated the diversity of possible relaxation mechanisms in their studies of manganese substituted carbonic anhydrase, showing clearly the contributions of both exchange and dipolar mechanisms by measuring the T_1/T_2 ratio and deducing the stereochemistry of inhibitor binding using the Solomon-Bloembergen equations.

After strictures concerning the undesirability of substituting manganese for zinc or magnesium, it was the turn of the naturally occurring iron sulphur proteins. The electronic properties of the iron in a series of ferredoxins from different sources was deduced from ENDOR and Mössbauer studies by Dr R. H. Sands (University of Michigan, Ann Arbor). The effect of iron and sulphur isotope substitution on the electron paramagnetic resonance spectrum of ferredoxins was also used by Drs J. A. Fee and G. Palmer (University of Michigan, Ann Arbor) to investigate the iron.

No Shining Peaks in Biochemistry

by our Special Correspondent

It looks as if 1970 will be a poor vintage for biochemistry; the Eighth International Congress of Biochemistry, held from September 3 to 9, had little to offer that really measured up to the grandeur of its surroundings in Switzerland. Many participants had already heard much of what was said and many others will have come away with a confused picture of what was new. For the more classical biochemists in Lucerne, the new models for photophosphorylation, the *entre nous* talk among mitochondriologists, and further advances in regulatory mechanisms were not enough to induce great excitement. The sessions on proteins, in Interlaken, showed a much more satisfying unity of approach and achievement, but to judge from the communications in Montreux, molecular biology is losing momentum, for much of the programme consisted of reviews of published work, and other announcements could confidently have been predicted.

DNA Polymerase and Membranes

The session on the replication of DNA started with a review by Dr J. Cairns (Cold Spring Harbor) of the explosive activity triggered off by his isolation of the *Escherichia coli* mutant which lacks the Kornberg DNA polymerase but can nevertheless replicate its DNA. Cairns cautioned against too uncritical an acceptance that the DNA synthesizing enzyme systems found in this mutant are indeed the true replicase(s) and pointed out that other of the accepted doctrines of replication are now rather less well established than they were when first propounded. The idea that both strands of DNA are synthesized in discontinuous fragments which are only subsequently joined together is probably wrong, for a detailed re-examination of this question using *Bacillus subtilis* has shown that the fragments anneal predominantly to only one side of the replicating fork.

If not proven to be the true replicating activity, the membrane systems which replicate DNA are certainly the most promising candidates to fill this role. Forebodings that these systems might comprise a complex structure of enzymes which could not be taken apart seem to have been premature because the replicating activity can be solubilized. Drs H. Schaller and F. Bonhoeffer (Tübingen) reported that their membrane system now replicates DNA in an *in vivo* like manner for up to an hour, an advance over the limited five minutes reported previously. Drs W. Stratling and R. Knippers (Tübingen) announced that they can solubilize the enzyme(s) of their membrane replicating system with deoxycholate. Interestingly, the solubilized enzyme(s) have different biochemical characteristics from the membrane system itself, and they too can work for up to an hour. The solubilized enzyme(s) are not inhibited by antisera to DNA polymerase.

An unexpected addition to the programme was the announcement of the finding of a second Kornberg DNA polymerase. Mr T. Kornberg (Columbia), the cellist son of Dr A. Kornberg (Stanford) who discovered the Kornberg DNA polymerase until recently thought

to be the replicating enzyme, has independently obtained a soluble enzyme activity from the Cairns mutant *E. coli*. The biochemical characteristics of this enzyme activity are similar to those of Bonhoeffer's membrane system and quite different from those of the Kornberg (senior) DNA polymerase. The new enzyme activity is ten times less sensitive to sulphhydryl reagents; it is completely inhibited by 0.2 M KCl (the original DNA polymerase is unaffected) and, most tellingly, it is not inhibited by antisera to the original DNA polymerase.

The structure of DNA also seems to be more complex than thought hitherto. By using DNA model polymers which contain identical base compositions but have different nucleotide sequences, Dr R. D. Wells (Wisconsin) has shown that the structure of DNA depends on its precise base sequence. When a polymer contains all purines on one strand and all pyrimidines on the other, its physical properties are different from a DNA of the same base composition but in which both types of base are intermingled on each strand. Poly dTG·poly dCA, for example, has a melting temperature six degrees higher than that of poly dTC·poly dGA. In all, five types of structure of DNA can be found. This finding provides direct experimental support for the hitherto theoretical suggestion that poly-pyrimidine runs in a natural DNA could comprise a recognition signal for proteins by virtue of its subtly different physical structure. Wells also reported the curious finding, based on X-ray and circular dichroism analyses, that poly dIC·poly dIC, a polymer with strictly alternating I and C on each strand, has a structure quite different from that of any other DNA; it seems to exist in an eight-fold left handed helix (other DNAs are right handed).

Transcription Factors

Some of the more interesting contributions came from speakers who tore up their abstracts to talk about more recent discoveries. These included two reports on ways to control transcription other than the production of a new σ factor. Dr A. Travers (Harvard) has found a new factor, the ξ factor, which directs *E. coli* RNA polymerase to transcribe ribosomal RNA. Unlike the σ factors, which control which messenger RNA species are initiated by the core enzyme polymerase, the ξ factor acts on the complete σ factor-core enzyme complex.

Development of bacteriophages has previously been thought to be controlled by the synthesis during infection of new σ -like factors which cause the host core enzyme to transcribe certain phage genes. This is certainly true for the T-even phages, but Dr M. Chamberlin (California) reported that *E. coli* cells infected with phage T7 contain a new RNA polymerase enzyme and not a new σ factor. This polymerase is quite unlike the host enzyme; it comprises a single polypeptide chain (the host enzyme is a complex of five subunits) which specifically transcribes certain T7

genes and does not seem to associate with the host enzyme at all.

Dr J. G. Gall (Yale) reported results obtained using the new technique of cytological hybridization by which the chromosomal position of satellite DNA has been located. The trouble with conventional hybridization is that it yields only quantitative data on the degree of complementarity between RNA and DNA: cytological hybridization gives more detailed information. Essentially, the technique is to prepare a tissue squash, denature the DNA, incubate it with a preparation of radioactive RNA or denatured DNA, and then locate the position of the hybrids formed by autoradiography. The answer obtained by hybridizing preparations of mouse satellite DNA, or the RNA which *E. coli* RNA polymerase transcribes from it, *in vitro*, to metaphase chromosomes is that these sequences are located in the short heterochromatic regions immediately adjacent to the centromere of each chromosome. Much interest was shown by questions about possible further extensions of the technique; two possibilities raised were that it might be possible to see whether the sequences of mitochondrial DNA are represented in the nucleus; and complementarity of the genomes of normal and transformed cells with RNA or DNA viruses could be studied. But although there is no objection to these experiments in principle, they would be very difficult technically.

In spite of the beautiful pictures shown by the crystallographers, the tertiary structure of tRNA is not yet resolved. Indeed, each new piece of research seems to demand the formulation of yet another model. Professor H. G. Zachau (Munich), who has managed to exclude several models by studying the availability of bases to reduction with NaBH_4 , launched an attack on the model builders which found support from Dr J. R. Fresco (Princeton). They agreed that it is of dubious value to construct generalized models from data gained from a single tRNA species, and ended with a plea for more experimentation and less speculation.

Proteins and Ligands

Now that structural resolution down to 3 Å is becoming routine, it is pleasing to observe how the X-ray crystallographers can stand face to face with the sequencers and kineticists and describe such a variety of systems in which protein conformation is influenced by specific complementary ligands. A session on triosephosphate isomerase, for example, provided an integrated picture of the enzyme from these three points of view based on a 6 Å crystallographic analysis by Professor D. C. Phillips (Oxford) which was "commissioned" for the congress. The same symposium included the 2.8 Å analysis of lactate dehydrogenase by Dr M. G. Rosmann *et al.* (now reported in *Nature*, **227**, 1098; 1970), but the discussion of these results was inhibited not a little by the fact that Drs W. S. Allison and N. O. Kaplan (San Diego) were not as far advanced with their sequence analysis as had been hoped. The power of transient kinetic procedures was illustrated by Professor H. Gutfreund (Bristol) and Dr T. Keleti *et al.* (Budapest) in studies on NADH binding and subunit interaction in heart and muscle LDH. Dr J. J. Holbrook (Bristol) pointed out that NAD-dependent dehydrogenases are not all "SH-enzymes" and no single structural feature—like, for example, the serine of hydrolases—can yet be implicated at the active site.

For the respiratory protein enthusiasts, there were most stimulating advances in elucidating the structure and function of erythrocyte cytochromes. Drs R. E. and R. Benesch and R. D. Renthall extended their previous finding that certain phosphates, and particularly 2,3-diphosphoglycerate, diminish the oxygen affinity of deoxyhaemoglobin. They showed how affinity labelling with pyridoxal phosphate can reveal the location of the site in the central cavity where the cofactors DPG and PLP bind. Dr M. F. Perutz proposed a stereochemical mechanism which explains haem-haem interaction, the alkaline Bohr effect and the regulation of oxygen affinity by DPG. This also provides a general model of cooperative interaction in allosteric proteins.

One of the most fascinating new problems in muscle biochemistry is the identity and function of troponin, discussed by Dr M. C. Schaub and Professor S. V. Perry (Birmingham) and also by Professor S. Ebashi—who, however, had been unable to proceed as quickly as would otherwise be expected of him because of the student unrest in Tokyo. On its own the interaction of myosin and actin cannot bring about contraction, and troponin seems to have a regulatory function in this process. Elegant solutions were also offered to two problems of longer standing: Dr Susan Lowey (Boston) spoke about myosin and Dr Carolyn Cohen offered a convincing interpretation of the structural principles of lattices involving paramyosin.

The membrane sessions were less uniform in topic and also in quality. Few participants were convinced by the idea of mini-proteins as the structural unit, but the transport binding protein discussed by Professor A. B. Pardee (Princeton) and Dr D. L. Oxender seems to be a tangible and discrete entity whose X-ray diffraction analysis is now being undertaken by Dr R. Langridge. The subject of Pardee's study is the sulphate transport system of *Salmonella typhimurium* developed by Dreyfus. Pardee likened the state of the art in this field to that of enzymology a couple of decades ago. But the isolation of about a dozen such proteins, their crystallization, and the finding that they can constitute up to 10 per cent of the membrane lend respectability to the subject. Drs W. Boos and A. Gordon, moreover, reported a finding which may represent a secondary phase in the history of the discovery: they have isolated two forms of a galactose binding protein from *E. coli*, possibly corresponding to different conformational states, with a 100-fold difference in binding affinity.

Antibodies and Clones

The excitement in this session was chiefly generated by the studies on antibody biosynthesis, although the structural and crystallographic aspects were not forgotten. One of the highlights was the superb technique reported by Drs A. R. Williamson, B. A. Askonas and B. E. G. Wright (MRC, Mill Hill) who have succeeded in selecting for a line of specific antibody producing cells which seem to be monoclonal in character as judged by the homogeneity of the product. Studies on the amino-terminal sequences of a number of myeloma and pathological immunoglobulin chains also evoked much interest among the immunopathologists, many of whom had just come down the valley from a meeting at Grindelwald. Finally, it was good to hear Professor M. Heidelberger discussing his recent work: he is now in his sixty-third year of immunochemical research.

The Closed Circuit—a Record of Soviet Scientific Life

In May this year Dr Zhores A. Medvedev, once head of the Department of Molecular Radiobiology at the Institute of Medical Radiology, Obninsk, was detained by the Soviet authorities in a psychiatric hospital, but was released in mid-June after protests from several distinguished Russian scientists. This account of Dr Medvedev's earlier unsuccessful attempt to accept an invitation to speak at the Ciba Symposium on Ageing at Sheffield in September 1966 is taken from his book *The Medvedev Papers: The Plight of Soviet Science*, due to be published in English early in 1971 by Macmillan and Co. (and St Martin's Press, New York, in the United States). The Russian text was published by Macmillan this week. The circumstances of this publication are recounted elsewhere in this issue.

My first visit to the Ministry of Health of the USSR showed that the attitude of the Foreign Section towards my trip to England had undergone a sharp change. The Deputy Head of this section, M. A. Akhmetelli, began to talk very vaguely about the fact that the Agreement on Cultural and Scientific Exchange with England had not yet been signed for 1966, and hence it was not clear how many man-days their section would have for official visits in 1966. Naturally, he said, if the plan for man-days in 1966 was not very large, then they would be able to arrange only the most important trips, agreed at the very highest level. Another rank and file employee of the same section, who had previously been given the materials from our Institute for safe keeping, hunted in his papers but could not find the file with the correspondence about the lecture and the resolutions of his relevant superiors. This file, which he had formerly had in his possession, seemed to have disappeared somewhere. I encountered the same lack of certainty in the Academy of Medical Sciences. Furthermore, it was necessary to start preparing an Exit Dossier again. The forms for the Exit Dossier are considered to be strictly accountable papers and are issued by the Foreign Sections of departments only to individuals whose trip has been agreed upon by the department. An Institute cannot prepare Exit Dossiers for capitalist countries on its own initiative.

After waiting a couple of weeks without obtaining any clear-cut decision, nor any official instructions to the Institute to prepare an Exit Dossier, I let Dr Wolstenholme know fairly clearly about the uncertainty which had arisen. I told him that the process of getting this kind of trip approved was a multi-staged one and that a first favourable reaction might be changed at various levels on its way to an actual decision. I could not tell him straight out the nature of the difficulties which had arisen, all the more so because I still did not know precisely what they were. It was necessary to speak in hints.

The process of arrangements here could be compared [I wrote] with multi-stage chromatography and ion-exchange. Imagine a set of columns with different

absorbents and ion-exchangers. A definite substance must pass through all of them. Those molecules which have no (+) or no (-), which are chemically inert, have more chances.

Anyone acquainted with chemistry and biochemistry, and this must include Dr Wolstenholme, would understand that no compound could pass through a system of both cation and anion exchangers if it had either a positive charge or a negative charge or both types of charges simultaneously on different groups. Only substances which are completely neutral, uncharged, without pluses or minuses, can get through.

I am not completely certain [I wrote] that I can by my own efforts ensure my trip to England, nor whether the relevant people will consider the personal invitations which I have received. Taking into account all the existing difficulties, I want to warn you that my trip to England is still problematic. There are two courses open to you to ensure the traditional lecture on ageing: first, to invite some other scientist whose personal wish and acceptance is sufficient guarantee of his attending the Symposium in Sheffield; or, secondly, to transfer the discussion of matters relating to this lecture to the official level, which would apparently have to be not lower than that of the Minister of Health. . . .

Again, as in 1960, I did not want to have to take the sins of others upon myself. If the responsible authorities did not reach a favourable decision, then let them conduct the correspondence and let them take the responsibility for the breakdown of this matter. In all events, I would do my duty, the lecture would be written, translated into English and sent to England, whatever the situation. The tenth traditional annual lecture on the problem of ageing would take place, whether or not I myself could go to England. With this aim, I began to prepare my first draft of the lecture; it was still too long, but I proposed to cut it later, keeping what was most significant. The lecture could not last more than seventy to eighty minutes.

The programme which I had received earlier was a preliminary one, which had been sent out only so that those attending could fix their plans for going to Sheffield in September, particularly since half of them were from other countries. The programme had not yet been published, and the Ciba Foundation would still be able to ask some other scientist to write the lecture. But, to speak frankly, I was sure that Dr Wolstenholme would choose the second course and would procure the visit of the lecturer he had already invited by top-level discussions. A short time later, I received confirmation of this. Dr Wolstenholme had drawn up an official letter to the Ministry of Health of the USSR, and had sent copies of his letter to the Director of our Institute and to me. The letter read as follows:

Professor B. V. Petrovskii,
Ministry of Health,
Rachmanovskii, Str. 3,
Moscow,
USSR.

Dear Professor Petrovskii,

It is my pleasant duty to write formally, on behalf of the Trustees of the Ciba Foundation, to ask you to give your official approval and assistance to enable a distinguished Russian scientist, Dr Medvedev, to come to England to lecture about his research.

The Ciba Foundation in London is an independent scientific and educational Trust which has been working in the field of international cooperation in medical research for the last sixteen years. We are assisted in this work by eminent scientists in many countries, who serve as our Scientific Advisory Panel. The representatives for the USSR are Academicians V. A. Engelhardt, A. I. Oparin and M. M. Shemyakin. Any one of these scientists would undoubtedly be willing to give you further information about the activities of the Ciba Foundation.

Some ten years ago, as part of our programme of medical research, we made a special effort to encourage fresh research, internationally, in relation to the problems of ageing. We have held a number of conferences on this subject, and each year we invite a distinguished scientist to visit England and lecture about his own special line of research in relation to ageing processes.

We now wish to invite Dr Zh. A. Medvedev (Chief of the Laboratory of Molecular Radiobiology, Obninsk) to give the Ciba Foundation's tenth Annual Lecture on Ageing Research. We should very much like him to tell us about his work, which we believe to be of international importance, and we should like him to do so in conjunction with a symposium on Ageing Research. This symposium will be held in Sheffield early in September 1966 and is being organized jointly by the Ciba Foundation, the Society for Experimental Biology and the British Society for Research on Ageing. We hope that Dr Medvedev will come and take part in this five-day symposium, with his own lecture (on the second day) being the main function of the week. Preliminary letters have been sent to Dr Medvedev and we have ascertained his willingness to prepare a lecture on his special subject for this occasion. We now hope that his visit to England can be given official sanction.

The Ciba Foundation undertakes to provide all the necessary travelling expenses, accommodation and living expenses for Dr Medvedev in connexion with this visit to England.

A copy of this letter is being sent to Professor G. A. Zedgenidze (Director of the Institute of Medical Radiobiology of the Academy of Medical Sciences in Obninsk).

We should be most grateful for your early, favourable attention to this matter, which we believe will strengthen the mutual respect and cooperation of Soviet and British scientists.

Yours sincerely,

G. E. W. WOLSTENHOLME
OBE, FRCP, FIBiol.

Three weeks after receiving this letter, I was summoned by telegram to see the Deputy Head of the Foreign Section of the Ministry of Health, M. A. Akhmetelli. I went in haste to Moscow, almost sure that Dr Wolstenholme's letter had produced the necessary action. By this time I had read up about the wide scale of the Ciba Foundation's international activity, and I knew that the USSR received considerable benefits from this organization, particularly in the field of public health.

My hopes, however, were unfounded. Akhmetelli did not receive me in too friendly a manner. He told me that the Minister of Health had decided against my trip to England. Akhmetelli hinted quite clearly that the English had done me too great an honour and that I ought not to be striving so hard to bring it off. When I asked who, in his opinion, was more worthy for such a mission, Akhmetelli was evasive. Akhmetelli also said that it was not quite convenient for the Minister to send a refusal to Dr Wolstenholme's letter, and that therefore I should send a reasonable refusal to England. If I did not do this, then their Section would hardly be able to do any serious business with me in the future. This was an obvious, although polite, threat, and it was only left to me to tell Akhmetelli my candid opinion of him and his department.

A few days later, the Director of our Institute was summoned to the Ministry. He was advised, in a more official manner, to send a short letter to Dr Wolstenholme, which would save the Minister from having to deal with the problem. I did not learn about this until later, when Dr Wolstenholme sent me a photocopy of this letter and a copy of his own reply to it.

The refusal, written in good English, read:

9.3.1966.

Dear Professor Wolstenholme,

I was very pleased to receive your kind letter in which you ask my assistance to enable Dr Zh. Medvedev to come to England to lecture about his research.

I express my regret, but I suppose Dr Zh. Medvedev will not be able to come to England this year because of a great pressure of work he has to do in his laboratory.

Yours sincerely,

Professor G. A. ZEDGENIDZE

Director of the Institute of
Medical Radiology of the Academy of
Medical Sciences of the USSR,
Member, AMS of the USSR.

Dr Wolstenholme's reaction was almost instantaneous.

Dear Professor Zedgenidze,

Your letter of 9th March comes as a very great disappointment not only to all of us at the Ciba Foundation, but also to the many people gathering in Sheffield in September who were particularly looking forward to an opportunity of learning at first hand about this work on Molecular Aspects of Ageing.

Dr Medvedev was also invited to the International Congress of Gerontology in Vienna, but had decided, correctly, that the lecture and meeting in Sheffield would give him a better opportunity to have the work discussed by appropriate scientists. It is a very great

pity if the work cannot now be heard either in Vienna or Sheffield.

So far as the Ciba Foundation is concerned, this annual lecture has been given by people from the USA, France, Holland, Israel, etc., and it will be a matter of very great regret to us if the USSR is not to appear in this series.

Since Dr Medvedev would have to be in this country for only one week, and indeed a visit of 2-3 days would certainly be better than nothing, is it really impossible to spare him from his duties for so short a time, particularly as this is presumably at a time when he might be on vacation?

I should be most grateful if you would reconsider the matter, and hope that there may still be the possibility of a favourable reply.

I am sending copies of this letter to Professor Petrovskii at the Ministry of Health, and also to Academicians Engelhardt, Oparin and Shemyakin who represent the Ciba Foundation in your country.

Yours sincerely,

G. E. W. WOLSTENHOLME
OBE, FRCP, FIBiol.

Together with the copy letters sent to Engelhardt and the other two academicians, Dr Wolstenholme wrote to each of them asking him to use his influence to obtain a favourable decision about the Ciba Annual Lecture.

Academician Oparin, as might have been expected, completely ignored the request and did not even answer Dr Wolstenholme's letter, but both Engelhardt (Director of the Institute of Molecular Biology) and Shemyakin (Director of the Institute of the Chemistry of Natural Compounds) actively sought to do what had been asked. I found out about this much later when Academician Engelhardt's campaign was over and he sent me copies of the correspondence on this matter and told me of the measures which had been taken.

At the end of March, Engelhardt and Shemyakin had prepared a special memorandum to the Ministry of Health

of the USSR and, having obtained an interview with the Minister, they handed it to Professor Petrovskii personally, urging him to take a favourable decision. It ran as follows:

Dear Boris Vasil'evich,

Please permit us to turn to you with a request that you take note of the question of granting to the Head of the Laboratory of Molecular Radiobiology at the Institute of Medical Radiology in Obninsk, Dr Zh. A. Medvedev, the opportunity of taking part, as principal lecturer, in the International Symposium on the Biology of Ageing to be held in England in the autumn of this year.

The Symposium is being held by the scientific organization 'The Ciba Foundation'. We are both members of the Council of this organization, as representatives of the Soviet Union. This Council includes scientists from a great number of countries, since the Ciba Foundation is of an international nature. It is a very solid organization with a high international repute. It undertakes a broad range of scientific and organizational work, it holds many specialized conferences and publishes a large number of monographs and collections of papers. It serves as an active centre of scientific contact between representatives of different branches of medical science, with scientists from related fields—chemists, biophysicists, pharmacologists, etc. In particular, one of the fields to which the Ciba Foundation pays special attention is gerontology, in its various aspects. The Symposium we are concerned with is the next event of the current year in this line. Preparations for the Symposium began over a year ago, and the organizers nominated as the principal lecturer, to give the annual lecture, Zh. A. Medvedev, the author of a number of important papers on problems of the biological principles of ageing.

However, at present, the situation regarding Zh. A. Medvedev's trip to England has taken a considerable turn for the worse.

The Director of the Ciba Foundation, Dr Wolstenholme, has approached us, as members of the Advisory Panel, with the request that, if possible, we should lend our efforts to prevent the breakdown of this long-planned Symposium, since the Annual Lecture is the central point of the whole programme.

In asking you to reconsider this problem, we should like to stress that the Ciba Foundation has always shown great courtesy to Soviet scientists, inviting this one or that to take part in its activities. Our scientists frequently visit the Ciba centre, where there are excellent facilities for scientific work, and avail themselves of the various forms of assistance offered by the Ciba administration. The difficulties which would arise out of a refusal to contribute to the success of this symposium would certainly not be in the interest of the expansion and strengthening of our scientific contacts abroad.

Academician V. A. ENGELHARDT
Academician M. M. SHEMYAKIN

The Minister of Health certainly paid attention to this letter, and turned his attention to the question it raised, but on quite a different plane. I was summoned again, this time by the Head of the Foreign Section of the Ministry of Health (this Section for some reason calls itself, in the list of Sections, the External Relations Section), Comrade Novgorodtsev, who advised me, in a categorical and high-handed manner, to stop all correspondence and activity connected with taking part in the Symposium.

Clearly, the trip could be written off as impossible. However, I decided "no surrender", and I tried to solve the problem one way or another. There were still four months left, quite long enough.

I wrote once again to Dr Wolstenholme, telling him that



Zhores A. Medvedev.

the Ministry of Health still had not reconsidered their decision. "Since," I wrote, "the programme of the Symposium has already been fixed and since my lecture forms part of that programme, then whatever the circumstances the Ciba Foundation will receive the text of this lecture in English not less than two weeks before the Symposium." However, I told Dr Wolstenholme, it would clearly be advisable to attempt another try, this time a contact between the Ciba Foundation and the Chairman of the State Committee on Science and Technology, who, being the Deputy Chairman of the Council of Ministers of the USSR, was on a still higher level, and was fully empowered to consider and reconsider any decision of this kind.

I no longer believed in the success of such a contact, but the experiment had to be made, all the more since the Head of the State Committee was Academician V. A. Kirillin who, back in 1960, had been in charge of the Science Section of the Central Committee of the CPSU, and later had been a Vice-President of the Academy of Sciences of the USSR. In 1963, as Vice-President of the Academy of Sciences, he had read my manuscript on the history of the genetic controversy, and, so I was told, had been favourable to it.

It was less than two weeks before I received from Dr Wolstenholme a copy of his letter to the State Committee on Science and Technology.

Dear Academician Kirillin,

I have the honour to address you as Head of the State Committee on Science and Technics of the Council of Ministers of the USSR, and would greatly appreciate your kind consideration of the following matter.

In March 1965 I wrote formally, on behalf of the Trustees of the Ciba Foundation, to Dr Zh. A. Medvedev (Chief, Laboratory of Molecular Radiobiology, Institute of Medical Radiology, Obninsk) to invite him to give the tenth in a series of annual lectures on research relevant to the problems of ageing, which are given by people of international importance. . . . The lecture [is to be] part of an international symposium . . . concerned with many aspects of the problem of ageing. Dr Medvedev, from his personal point of view, kindly accepted our invitation. . . .

Dr Wolstenholme then briefly outlined the history of his correspondence with the Minister of Health and the Director of our Institute, and also explained the problems and aims of the Ciba Foundation. At the end of his letter he wrote:

It is our strong wish to encourage wider recognition of Soviet scientific achievements, and, at the same time, to do everything possible to improve official and personal friendship. We greatly hope, therefore, that you would be so courteous as to use your influence to overcome whatever minor obstacles there may be which so far prevent Dr Medvedev from confirming his ability to give our lecture and participate in the international symposium in Sheffield.

Academician Kirillin used his influence, but, like the Minister, in an entirely different sense from what Dr Wolstenholme had hoped for. At the end of May, a member of the State Committee for Science and Technology, D. Pronskii, who was Head of the International Section of this Committee, forwarded Dr Wolstenholme's letter to the Ministry of Health of the USSR, with a covering letter of his own. In this letter, he stated insistently and in an insulting manner that Zh. A. Medvedev had broken the regulations about "intercourse with foreign

firms" and recommended that these regulations should be explained to Medvedev. Judging from this letter he did not even understand that the Ciba Foundation was not a firm but was a scientific organization. But, nevertheless, his letter found the necessary response in the Ministry of Health.

In the middle of June, I was summoned by the Head of the Special Section of our Institute, and, on the instructions of the Director, I was made familiar with a letter (of 10 June 1965) which had come to our Institute from the Head of the Foreign Section of the Ministry of Health, Comrade Novgorodtsev, to which was attached a copy of Pronskii's letter to the Ministry of Health. Repeating in part the expressions of Pronskii, Novgorodtsev wrote in his letter that Zh. A. Medvedev had "broken the regulations about correspondence with foreign firms", that "he was striving to make this trip by any means, and was involving scholars who had nothing to do with it". Here he had in mind Engelhardt and Shemyakin. Further, Novgorodtsev expressed his doubt that Medvedev was sufficiently competent in the problems which it was proposed he should raise in his lecture, and he also recommended that the necessary administrative measures should be taken to let Medvedev know that his behaviour and his position of opposition to the decisions of the Ministry were not acceptable.

It should be noted that Novgorodtsev's letter was sent to the Institute via the Special Section deliberately, for the psychological effect. A copy of this letter from the Ministry of Health was also sent to the Academy of Medical Sciences of the USSR.

Ten days later, via the same route, an enquiry came from the International Section of the AMS, signed by a new head of this section, Academician Kovanov of the Academy of Medical Sciences. In this letter, he told the Institute to let him know what concrete measures had been taken by the Party Organization and the Institute authorities about Zh. A. Medvedev, in connexion with his breach of the rules on correspondence and contact with foreigners, and a reference to Novgorodtsev's letter on this matter followed.

The Special Section of our Institute demanded an explanatory memorandum from me on this matter. I wrote them one, clearly pointing out the misunderstanding which had arisen both in the Academy of Medical Sciences and the Ministry of Health. I also clearly indicated my opinion on Comrade Novgorodtsev's power to judge my competence in the field of gerontology. So far as I know, my explanatory memorandum was sent to the AMS and the Ministry of Health by the same channels which had brought their letters, but neither an answer nor a "sorting out" of the matter followed.

There was now little more than two months until the opening of the Symposium. The lecture was written, and the translation into English almost finished. At the end of July, my old friend Ralph Cooper was arriving for the International Microbiology Congress in Moscow. I was counting on him to help me edit the translation of the text. Dr Cooper and I had worked together in the biochemical laboratory of the Timiryazev Agricultural Academy in 1958-59, when he visited the USSR under a scientific exchange programme. He was at that time a young biochemist who had just finished his postgraduate course at Oxford and had been working for about a year at the Rothamsted Experimental Station. When he arrived in Moscow, not knowing Russian, he was almost helpless, particularly since our working conditions are so different

from those in England. He was at first assigned to the Department of Microbiology, but then he was transferred to our Department of Agrochemistry and Biochemistry. I shared a small study with him, and in the end we used to coach each other in our respective languages. During the year, we became good friends, and afterwards we had kept up a constant correspondence. In 1961, Ralph Cooper was a biochemistry lecturer at Hatfield College.

Ralph Cooper was coming to the Microbiology Congress with his son Paul, who was twelve years old. During the Congress Paul was to stay with us in Obninsk, with my sons, the elder of whom, Sasha, could speak a little English.

At the beginning of July, another letter came from Dr Wolstenholme.

Dear Dr Medvedev,

... We have received no further news from you or from the Soviet Authorities, and greatly hope that in this interval arrangements are going ahead favourably for your visit to give this important lecture for the Ciba Foundation, and for your participation in the very interesting symposium in Sheffield. I leave for my holidays on 28th July and will be back in England only a day or two before the Sheffield meeting, so that I hope there will be good news before I leave. In any case my office will be in touch with me while I am away.

A room is being held for you at the Ciba Foundation for the nights of 2nd and 3rd September. ... A room has also been reserved for you in Sheffield ... for the nights of 4-9th September inclusive. ...

If the worst comes to the worst, and you are refused permission to come to England—although I cannot imagine what valid reason there could be for this—then we should hope to receive your manuscript, with any slides, in good time so that we could arrange for Dr Strehler to present the lecture for you.

By this time I had already sent the Russian text of the lecture, some forty pages, for the necessary official approval, without which not a single post office in the country would accept it for dispatch. The procedure for such approval had hardly changed at all since 1960. Obtaining approval for posting, with the special form 103A, required the preliminary consideration of the text by the Academic Council (with two representatives), a decree of the Commission that it was non-secret, and decisions by a certain department of the Academy of Medical Sciences of the USSR, the International Section of the AMS and Glavlit. After permission had been granted for the text to be sent abroad, the English translation had to be considered, and the Institute itself had to verify that this was identical with the Russian text.

The failure of the attempt to get a reversal of the previous decision on the trip by way of the State Committee for Science and Technology left me only one possibility of further action—to go to the Central Committee of the CPSU, the final and highest of all departments, for all decisions on foreign travel to capitalist countries.

The Secretariat of the CPSU, unlike all other bodies, has the right to send people abroad for short periods, even at short notice. I knew of a case of a footballer who was suddenly required for an international match; he was summoned and rushed by air from the resort where he was on holiday, approved by all departments, including the visa section, delivered from Moscow to England, driven straight to the stadium from the airport, and all this within twenty-four hours. He was to play for the Rest of the World against an All-England side. The idea of the match had come up unexpectedly, and no preparations had been

made for it. So far as I remember, England won. But this, of course, was a special case; football, sport, the glamour, the prestige! No doubt the Chairman of the Exit Commission watched the match himself in the English stadium or at home on TV. It was not a lecture on gerontology.

A preliminary discussion with some employees of the Central Committee of the CPSU, mainly by telephone, led me at last to the section which had it in its power to be most helpful in the solution of the problems which had arisen in contact with other departments. This was the International Section for Capitalist Countries of the CPSU. (There was yet another International Section which was responsible for problems connected with socialist countries of the East and West.)

At the very end of June, I was received by the First Assistant to Comrade Ponomarev, the Secretary of the Central Committee of the CPSU who was in charge of this Section. Comrade Ponomarev himself was away at this time, accompanying the French President, De Gaulle, on his tour of our country. His assistant, Comrade V. S. Shaposhnikov, met me in a most friendly manner, and our conversation lasted more than an hour. He was interested in gerontology, its achievements and the reason why our country was lagging behind in a number of directions in biology. He had made himself thoroughly familiar with my case; he promised to take all necessary measures to set the matter right and make the visit possible, and, in case of necessity, to interest Comrade Ponomarev in the matter personally. After this visit to the Central Committee of the CPSU, I had the first feeling of hope that something would be done and the trip to England would take place.

However, no results were apparent in the course of the next two weeks. When I received Dr Wolstenholme's letter, quoted above, I once again applied to Comrade Shaposhnikov, in writing, and at the same time sent him a photocopy and translation of the letter from England. In a telephone conversation, Shaposhnikov said that he was very sorry but he had not yet had time to deal with my business, but he promised once again to do everything possible in the very near future.

At the end of July, my friend Ralph Cooper arrived in Moscow. Naturally, I told him about my problems, and, when we had considered the situation carefully, we decided that my best plan would be to send a copy of the English translation of the lecture by him, for safety, since the approval to send it by official channels was taking so long. The International Section of the AMS had sent the manuscript of the lecture first for review to the Institute of Gerontology in Kiev, and Glavlit, which is not to be hurried, was still to come. Cooper promised that when he got home he would edit the translation from the language point of view, have it retyped if there were a lot of corrections, and deliver it to the Ciba Foundation by mid-August. He would send the copy of the lecture to Dr Strehler. Bernard Strehler was taking part in the Symposium and I asked him if he would read my lecture in case I could not go myself. I knew from the Biochemistry Congress in Moscow in 1961 that Strehler was a very fine speaker and could carry out this task excellently.

To take advantage of a convenient opportunity such as this for sending the text of a lecture was not, of course, in the bureaucratic sense, in accordance with the "rules for intercourse with foreign firms". But no other way out was left to me. If I could not get the text of the lecture to my English colleagues in good time, I should be to blame in their eyes, I should have defaulted. No

reasonable Englishman could ever believe in the reality of the procedure for sending a scientific text which I have described above. He would not believe it, but would think that I had made it all up to cover the fact that I had been unable to write the lecture, although I had already written to England that the lecture was now ready.

The course of subsequent events proved that I had acted correctly. The official procedure (and this only under pressure from me) was as follows. The report from Kiev, from the Institute of Gerontology, was received in August. Fortunately, the Kiev experts did not recommend any changes. Glavlit's permission to send the lecture abroad was granted on 4 September, and the text of the lecture was sent to England on 5 September, the day before the session when the lecture was to be delivered. It was received in England on 11 September, when the Symposium was already over.

In 1939, when Nikolai Vaivlov was refused permission to go to Scotland for a Genetics Congress of which he had been elected President, he sent the text of his speech unofficially via his Bulgarian friend, Doncho Kostov, who was going to the Congress from Leningrad. At that period, Vaivlov was risking far more than I was. I regard this as an example for scientists to follow, and in this case I find no moral problem in breaking rules when they have become ridiculous.

In the middle of August, I received from England several leaflets announcing my lecture. These leaflets were being distributed to the science centres, universities and colleges of Great Britain. They announced in large type:

THE 10th (ANNUAL)
CIBA FOUNDATION LECTURE
ON
RESEARCH ON AGEING
WILL BE GIVEN BY
DR Z. A. MEDVEDEV
(USSR)
SUBJECT:
"MOLECULAR ASPECTS OF AGEING"
on Tuesday, 6 September 1966, at 8.15 pm
in Lecture Theatre No. 1, University of Sheffield
Chairman: Professor H. N. Robson
Open without fee or ticket to all interested

It was not open only to the lecturer himself.

At the same time as he sent the leaflets, the Deputy Director of Ciba told me that as a final means of ensuring my trip to England, they, together with the British Society for Cultural Relations with the USSR, had approached the Soviet Embassy in London and asked for their help, and had written to the British Embassy in Moscow, so that, in case the Soviet authorities did request a visa for my visit to England, it could be issued without the delays in correspondence usual in such cases.

Once again, I sent all this material and one of the leaflets to Comrade Shaposhnikov, enclosing a covering letter. There was, however, no answer.

At the end of August, a letter came from Ralph Cooper. He told me that the correction to the text of the lecture

had been completed and that it was now in the hands of Ciba.

The Deputy Director of Ciba [he wrote], Dr de Reuck, sent a telegram to Bernard Strehler, to confirm that he will be present. He also suggested that I should go to Sheffield as your representative, and, if necessary, give an explanation of your absence. Thus everything necessary has been arranged. . . .

September drew near, and my hopes of being able to make the trip finally died. Comrade Shaposhnikov was away on leave, and it was now too late to start another round of high-level discussions. I began to be very concerned about the absence of any legal guarantees of so important an aspect of the rights of man and of the scientist which the freedom to travel abroad and to engage in international cooperation is recognized throughout the world to be. When it was clear to me that the lecture would take place without its author, I decided to send a special explanation to England, making the reason for my absence clear. I prepared this "explanation" six days before the lecture, and sent it from Moscow, from the International Post Office, by registered express airmail, requesting an "advice of delivery" and sending it, for safety, to three people: Dr Wolstenholme, who was by now in Sheffield; the Chairman of the lecture, Professor Robson; and Ralph Cooper. I asked them to read this text either before or after the lecture, whichever was more convenient. I was not sure whether or not these letters would get through to England, but, as it turned out, only one of them went astray. The other two reached Sheffield on 5 September, and the "advice of delivery" notes were returned to me.

And so came the day of 6 September, a day which should have been a day of triumph for any scientist. Early in the morning, I set my watch to British time. My lecture was to take place at the evening session. The morning was given over to two sessions on problems of the ageing of plants. In Obninsk, however, a sterner task awaited me.

In the autumn, as is well known, all city organizations take part for a couple of months in the potato harvest. It just had to happen that the turn of our section, that of Radiobiology and Genetics which comprised four laboratories, to go potato picking came precisely on 6 and 7 September. That morning, with my colleagues from our laboratory, I travelled 25 kilometres by bus, out to the State Farm. In Sheffield, they were just getting ready for the first morning session, someone else was in the Chairman's seat instead of me, while we were carrying baskets and starting to sort the potatoes, moving back and forth along the furrows. The section was assigned to a field of some two hectares. The potatoes had to be collected, sorted into large and small, and the large ones loaded into the backs of the lorries. The small ones went to the farmyard. We finished our working day at around four o'clock. By this time the second morning session in Sheffield was over.

The dinner in Sheffield was at 18.30, British time. In Obninsk, I had a few of my friends coming to my home at this time. The dinner in Sheffield did not last long, the lecture followed it. But we, in Obninsk, had no need to hurry, and we sat over it, talking. When, by my reckoning, my explanation might well be being read in Sheffield, I too read it aloud to my own guests at home.

Translated by Vera Rich.

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Cost of Preventing Thames Tidal Flood

by

A. N. SCHOFIELD

Department of Civil and
Structural Engineering,
University of Manchester Institute
of Science and Technology

Professor Schofield's appraisal of a recent Greater London Council report on ways of preventing the impending disaster in the flood plain of the River Thames includes the cost of risk of future loss. At present values, it appears economic to use sandbags immediately to raise the embankments.

In January 1968 the Greater London Council (GLC) was asked by the Ministry of Housing and Local Government to undertake "an urgent investigation" into the degree and form of protection of London from tidal flooding. As a result of this, the GLC, in October 1969, published its first report of studies on Thames flood prevention¹.

Although this report has been available to the public for some time, for example, in the library of the Institution of Civil Engineers, I only studied it recently when the GLC sent me a copy. My data are taken from the report, but in calculations I have followed the methods set out in the handbook *An Introduction to Engineering Economics* published by the Institution of Civil Engineers (ICE) in 1969. I am concerned with the urgency and the economics of protection needed for London; there is also academic interest in the manner in which time dominates the calculations for these projects.

If there were catastrophic flooding in Central London, the flood waters could wash from Acton Vale and Shepherds Bush across to Barnes Common; from Earl's Court and Buckingham Palace across to Clapham Junction, South Lambeth, Camberwell and New Cross Hospital; from Stratford, West Ham, Wallend and Dagenham across to the Plumstead Marshes. Levels in the flood plain through which the Thames meanders are well known and, if a tide overtopped the statutory defence level of the river walls, a two mile wide strip of low level ground could have water 10 feet deep in the streets in some areas. The GLC studies state that in the sixty-five square miles of the London Flood Plain 1.5 million people are at risk by day and 1.2 million people by night. The cost of direct damage is estimated at £1,000 million. Indirect costs are not estimated but are called "very great".

On pages 45-50 of the ICE handbook benefits and costs of works to prevent a flood are calculated as follows. The present value A of future losses due to floods which return after a period of m years and each time cause a loss L is approximately $A = L/mr$ where r is the annual rate of interest on capital. The handbook goes on to calculate the rising cost and diminishing risk associated with increasing the embankment height and thereby increasing the return period m . This note will attempt by similar calculations to appraise the various projects for prevention of flooding in London, assuming a rate of interest $r = 0.08$.

The GLC report is very interesting in its discussion of the return period m . An appendix 7 by J. R. Rossiter dated April 17, 1969, explains that in Central London the river's mean high water height is rising relative to the defences at a rate of 2.5 feet per century. C. T. Suthons in 1963² considered the frequency of occurrence of abnormally high tides. River levels observed in a surge tide on December 10, 1965, established the variation of high water height at various points along the river. These various studies are crystallized in Fig. 14 of the report which shows that in 1970 the flood to overtop present

defences returns every ten years on average; if the defences are raised 2 feet the flood return period increases to 100 years. The rise of high water heights will continually reduce return periods unless defences are also continually raised 2 or 3 feet a century.

Cost of Risk

The report's estimate of loss in the impending London Flood Plain disaster is based only on a comparison with damage to Hamburg in 1962, and the estimated £1,000 million is the least certain figure in these calculations. If we knew that this loss would occur in ten years and then recur regularly, we might allow for payments of $\pounds(1,000 \times 0.06903) m = \pounds69$ million every year into what Table 4 of the ICE handbook calls a sinking fund. If the loss occurs this year and we know it will recur regularly every ten years we might borrow money to pay for damage and service the debt by annual payments of $\pounds(1,000/6.7101) m = \pounds149$ million. To assess the annual value of the present risk without unwarranted sophistication of the calculations it seems reasonable to take a convenient figure somewhere between £69 million and £149 million, say, of the order of £100 million per annum. That annual cost will be included in appraisal of projects with various times to completion.

The original project that the report considers is a permanent increase of river wall levels by 6 feet, which would increase the flood return period a thousand times and reduce the risk to a small value. This may cost £65 million (Table 5, ref. 1) but is estimated to need thirteen years for completion (page 34). If every year for thirteen years there is £5 million of engineering cost and £100 million risk, then at present values such a project has the same present value as a 13 year annuity which, from the annuity Table 3 of the ICE handbook, costs $\pounds(105 \times 7.9038) = \pounds829.9$ million. The present value of subsequent risk is then small.

The "least time to provide flood protection including bank reconstruction of any scheme considered" is six years for a tide control structure at Woolwich which may cost £45 million. To calculate the present value of that project we could first calculate the present value of risk for the six unprotected years. The present value of some future cost or benefit is discounted by a factor $(1+r)^{-n}$ if the cost or benefit does not arise for n years, for in those n years the present money value could be earning interest at a rate r . Assuming a rate $r = 0.08$ and looking at the ICE handbook's Table 3 the present value of an annuity of £100 per annum for six years of risk is £462.3; the risk thus adds a cost of £462.3 million to the present value of this project. Assuming the tide control structure must be paid for as a single sum paid in 5 years time, from the handbook's Table 2, at present values its cost is $\pounds45 \text{ million} \times 0.68058 = \pounds30.6$ million. So at present value the cost of risk of loss and of engineering works is the sum of $\pounds(462.3 + 30.6) = \pounds492.9$ million. Alternatively for the same project we can calculate that every year for six

years there is £7.5 million of engineering cost and £100 million risk so at present values such a project costs $\pounds(107.5 \times 4.6229) = \pounds497$ million. The discrepancies between these two estimates for the same project indicate a measure of uncertainty within the calculations, but clearly the cost of the Woolwich project at present values is about half the cost of the original project at present values, and the Woolwich project seems more profitable to pursue.

This calculation emphasizes the insensitivity of present values of projects to the detailed engineering cost. The significant data are the estimated total loss, the return period of floods, the interest rate and the time for completion of works. To test the sensitivity of the calculation suppose that the loss is very much less, with an annual value of only £10 million. At present values the costs of the Woolwich project fall to $\pounds(46.7 + 30.6) = \pounds76.8$ million. To demonstrate the insensitivity of even this calculation to engineering cost, suppose that it increases by £10 million in five years, the present value of the variation is £6.5 million which is less than 10 per cent of the total present value even at that greatly reduced level of loss.

Alternative Proposals

It seems to be worth getting a detailed survey and estimate of probable flood plain losses: this action seems more likely to produce benefits by identifying reducible risks than an engineering design study is likely to produce benefits by reducing costs. If borough engineers were simply to have marks painted on the street lamps at the level of the statutory defences of the adjacent river, occupants of the flood plain could themselves attend to easily reducible risks and could advise their engineers of the value of less easily reducible risks.

If the engineering design study reduced construction time, that would be valuable. But six years is more or less what was quoted in the report from the Ministry of Housing and Local Government³, so no technical advance since then is evident. Suppose a proposal were made that could reduce construction time to three years. The present value of an annuity of £100 million/year for three years is £257.7 million: for six years it is £462.3 million. Such a proposal could therefore be worth $\pounds(462.3 - 257.7) = \pounds204.6$ million at present values.

An interesting comment occurs on page 38 of the GLC report. The disruption of shipping by intermittent action of a barrier was estimated to be equivalent to a present value of £10 million, and the sentence continues: "the river walls could be raised by about 2 feet for that sum". No time is assigned for that task but a similar task described in appendix 11 is raising banks below Woolwich in a period of three years. The cost is about the same, so I shall assume that the raising of river walls in central London by 2 feet will take three years. The risk costs £257.2 million as before. The construction cost in two years may be $\pounds10 \times 0.857 = \pounds8.6$ million in present value. In addition, in three years' time we must make allowance for the risk of loss with a flood that rises 2 feet above the statutory defences. Fig. 14 of the report shows that flood returns in 100 years. Our allowance against that risk of loss might then be a single sum such as the ICE handbook calculates of $A = L/m \cdot r = 1,000/100 \times 0.08 = \pounds125$ million. Discounting that to a present value of $\pounds125 \times 0.79383 = \pounds99.2$ million we see that we might allow for three years of high risk, raise the banks only 2 feet, and allow for a future risk for a sum of present value $\pounds(257.7 + 8.6 + 99.2) = \pounds365.5$ million. There is a

direct advantage over the Woolwich project of $\pounds(492.9 - 365.5) = \pounds127.4$ million and direct benefit in not interfering with any shipping.

Advantage of Sandbags

The great advantage of an early completion is clear. Wherever discounting methods are introduced it follows that project teams will introduce "critical path" methods and other planning and programme evaluation techniques that are directed towards early completion of design and construction. It seems on the face of the matter quite likely that the defences could be raised 2 feet by use of sandbags in a few months of emergency action. The cost of sandbag defences may be large, but perhaps it will be less than the £150 per foot quoted for embankment works in appendix 11 of the report; let us assume the cost is £50 million. Subsequently there could be alternative developments, but suppose we return to the original project and every year for thirteen years allow £10 million for risk and £5 million for engineering work, redeveloping the river frontages to new statutory defence levels above the present levels. At present values the preliminary sandbag defences and the embankment raising work only costs $\pounds(50 + 15 \times 7.9038) = \pounds168.6$ million, and one might well consider what costs of development of frontage could be met by the various categories of frontages.

The GLC report explains on page 34 that "at present the London Floods Acts are administered by the GLC and the frontager is responsible for the provision and maintenance of flood defences to a line and level to be determined by the statutory authority" who can "determine a new line and level and enforce the provision of flood defences by the frontager". But while the GLC has power to enforce a solution of this problem on the banks, it requires new powers and finance to solve the problem by works in the river.

At this point this appraisal of the GLC report may have been taken far enough. The data on which my economic evaluations are made are probably out of date, for they were all available in April 1969. Since then much scholarly work and wide consultation in many committees has clearly continued with urgency and dedication on the part of all involved. The report has proved to be full of interest and clearly merits academic attention: it is to be hoped that other publications will include full economic evaluation of alternative projects. The lesson of my economic evaluation is that this is an urgent problem that needs a crude solution. I am only too well aware of a tendency for engineers and scientists to want to "solve" problems like floods with solutions more elegant than sandbags. I am also aware, as the GLC report points out on page 45, that in development of amenities "the greatest restriction at present lies in the physical barrier between land and water created by the flood defences". But there is real risk to life, and when I adopt the economic criteria advocated in the ICE handbook my calculations emphasize the economic value of a timely physical barrier. At present, as we approach yet another winter when a gale in the North Sea may raise another high tide like that of 1953, the occupants of the flood plain have again to face an impending London flood plain disaster inadequately protected and unprepared.

¹ *Thames Flood Prevention Barrier/Barrage Project, First Report of Studies* (Greater London Council, 1969).

² Suthons, C. T., *Proc. ICE*, 25, 433 (August 1963).

³ *Technical Possibilities of a Thames Flood Barrier*, Cmnd 956 (HMSO, London, March 1960).

Game and Problem Structure in Relation to the Study of Human and Artificial Intelligence

by

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Programming a computer to play board games is an effective way of modelling a set of problem solving strategies. The analysis of games played against such programs is a convenient laboratory method for studying human intellectual capacities, including skills allied to social intelligence.

THE value of games for the study of human and machine intelligence is hardly in doubt, but it can be argued that the development of research in this area has been more haphazard than it might have been. This article therefore attempts to define those parameters of a limited subset of games—fully “rational” board games, which determine the characteristics and the complexity of the skills involved and hence the possible roles of different games in psychological research.

The evolution of board games, like that of other tools, has been primarily related to social pressures, the direction and pace of development being dependent on the general cultural background. Some of the evolutionary trends have, however, been consciously directed, and have involved deliberate experimentation. Thus Murray¹ attributes the development of modern chess from the mediaeval game to a European preference for a short intense struggle as opposed to the Eastern liking for a lengthier intellectual and strategic analysis which was the central characteristic of the Muslim game. During the period of change a number of possibilities were tried which included the reintroduction of dice, the enlargement of the board and the introduction of new pieces. In the end the only surviving changes of importance were greater powers of movement for the queen and the bishop, changes which incidentally greatly enhanced the value of the pawns because promotion now so increases the power of an attack that usually once this is achieved it becomes almost irresistible. These changes gave chess a new lease of life, increasing the speed with which the opposing forces could contact, hence shortening the opening phase, and speeding up the end game. Nevertheless, as an experimental tool, chess is still susceptible to criticism. Some of its staunchest supporters (for example, Good²) are at the same time the strongest advocates of reform.

Two of the chief complaints of the early European players were that the game was too long in coming to a head and that the best tactics for the opening play were not easy to discover and hence skill in chess was very dependent on rote learning. These weaknesses, though diminished, are still relevant and it is possible that chess is complicated in the wrong sort of way. Although recent computer based research and two classic non-computer

studies^{3,4} have made important contributions, it is, in fact, difficult to use chess as a method of analysing human problem-solving skills as such.

Board Games

In his book, *A History of Chess*¹, Murray divided board games into three groups: (1) race games, in which men are moved along a well-defined track; (2) hunt or siege games, in which one side attempts to block or confine the other; and (3) war games, which in the capture of prisoners plays a major part. In a later analysis⁵, Murray classified these games as (i) games of alignment and configuration; (ii) war games; (iii) hunt games; (iv) race games; and (v) mancala games. Bell adopts the same classification⁶, except that hunt games are classified as war games. A. E. and Jagoe⁷ have criticized this type of classification on the grounds that it does not provide mutually exclusive categories, and have suggested one in terms of the variables which determine the games' complexity. In developing this approach, this article treats board games as members of a subset of problems, which includes games, puzzles, psychological tests and the laboratory situations used by experimental psychologists. The unifying characteristic of this subset is that its members are all make-believe situations which act as paradigms for the reality problems of life. Puzzles are essentially single person games, psychological tests are sets of puzzles, and interviews and vivas are two-person games.

To illustrate this approach, the parameters which determine problem complexity for a number of naturally evolved two-person board games are listed in Table 1. Of these parameters only partiality may need explanation. According to C. A. B. Smith⁸, a game is impartial where, if the rules state that there is a legal move from a position (N') to a position ($N'+1$) this applies equally to both players. Either player, should it be his turn, can make this move. In impartial games therefore the pieces belong to neither player and the advantage depends solely on the parity of the move. Nim—but not Northcott's Nim—and the French Polytechnique game or “Boxes” are good examples of impartial games. Although, as Smith points out, such games are relatively simple to analyse mathe-

matically, they have the severe disadvantage that it is impossible for either player to establish any "rational" lead greater than the advantage of the move.

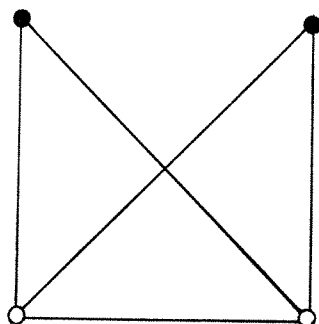


Fig. 1. Ko-No is played in the Punjab and China. Different "boards" are used in Korea and Thailand. Two players with two men move alternately along a line of the "board" to an adjacent point which must be empty. No captures can be made and the aim of the game is to blockade the opponent's men.

It should be clear from this type of analysis that games, and indeed problems in general, can gain in complexity either by an increase in the number of parameters or by an increase in the number of values or levels which the parameters can take. Chess and Wei Chi or Go are each games of considerable complexity which differ in having evolved in these two contrasting ways.

problems which are the operands of human thinking outside the laboratory.

In the past, psychologists have attempted to treat cognition and conation, that is to say intelligence and will, as separate functions, but it is now generally recognized^{9,10} that the broad distinction between cognitive tests and tests of other personality variables is a little blurred and artificial. Intelligence is essentially a synonym for biological adaptability or, more bluntly, the ability to get out of tight corners, and hence one can define two basic biological computational problems. First, the organism is often faced with converting its existing environment into one of greater safety. This is essentially Cannon's concept of homeostasis, which we can represent in an n -dimensional problem space either as a taxis or as a flight from A to B . Second, in the absence of an immediate threat the organism can optimize its survival by exploratory activity which enables it to understand or map the structure of its environment so that when a threat does arise it will be able to take the most effective evasive action.

The structure of any problem solver inevitably determines—or reflects—the characteristics of the problems which the organisms can tackle, and, because man's dominant perceptual input involves the representation of his n -dimensional problem space as a two-dimensional retinal map, it is convenient experimentally to devise problems in terms of the organization of the movement, across a two-dimensional grid, of pieces whose properties represented as a board game. Indeed, many of the psychological tests and model problems which have been de-

Table 1. SOME PARAMETERS WHICH DETERMINE THE COMPLEXITY OF EVOLVED BOARD GAMES

Parameter	Chess	Go	Boxes	Five-in-a-row	Northcott's Nim	Ko-No
Partial or impartial	Partial	Partial	Impartial	Partial	Partial	Partial
Aim	Capture of pieces	Capture of territory	Capture of territory	Alignment	Blockade	Blockade
Conventional size of board	8 × 8	19 × 19	$n \times m$	24 × 24	8 × 8	3 × 3
Complications of board	—	—	4 × 4 not trivial	—	—	—
Possibilities of varying size of board	+	++	+++	+++	+++	—
Conventional number of pieces	32	361	Variable	Variable	16	4
Variability of number of pieces	+	Varies as board	Varies as board	Varies as board	Varies as board	—
Type of piece: by move	6	1	1	1	1	1
by power	2	1	1	1	1	1
Types of move	8 (+2)	1 (entry)	2 (entry)	1	1	1
Capture of pieces	+	+	—	—	—	—
Method of capture	Replacement	Custodian	—	—	—	—
Promotion	+	—	—	—	—	—
Stacking	—	—	—	—	—	—
Stop rules	+	+	—	—	—	—
"Fairy" rules	+	—	—	(+)	—	—

Ko-No (Fig. 1) is probably the simplest fully determined board game known. It is also an unbounded game in the sense that the games tree contains loops which cannot simply be defined as draws. In terms of searching the complete games tree, Ko-No (Fig. 2) is clearly trivial, and the fact that, although unbounded, it is also a playable game strongly supports the view that in the absence of acquired heuristics man's capacity for logical analysis is relatively small.

Hierarchical Problem Sets

If, as we argue here, games are models for reality problems then it behoves us to define the real or biological

veloped for the study of human problem solving can be relatively easily represented as board games, and hence usefully included in this analysis. One example is a Perceptual Maze Test¹¹ originally developed for the study of frontal lobe functions; another is the Fungus-Eater game proposed by Toda¹². Travis¹³, in his experiments with a theorem using program, used the same approach and a basic puzzle or problems of movement A is B is defined later in this article. The value of developing problems of this type, that is to say with a systematic and hierarchical structure, has been discussed by the authors concerned and is fairly obvious. Moreover, as Travis emphasizes, the rules of a game form a defined calculus. The problem of sequencing operations within

such a calculus is identical with the basic logical process of constructing a derivation from a given premise through a sequence of permissible symbol transformations. To produce a solution B from premise A the simplest operation is the magical transformation $A \rightarrow B$. In the real world, as in games and with formal problems, there are always restrictions on the transformations which are permissible, and it is the characteristics of these restrictions which determine the complexity of a problem.

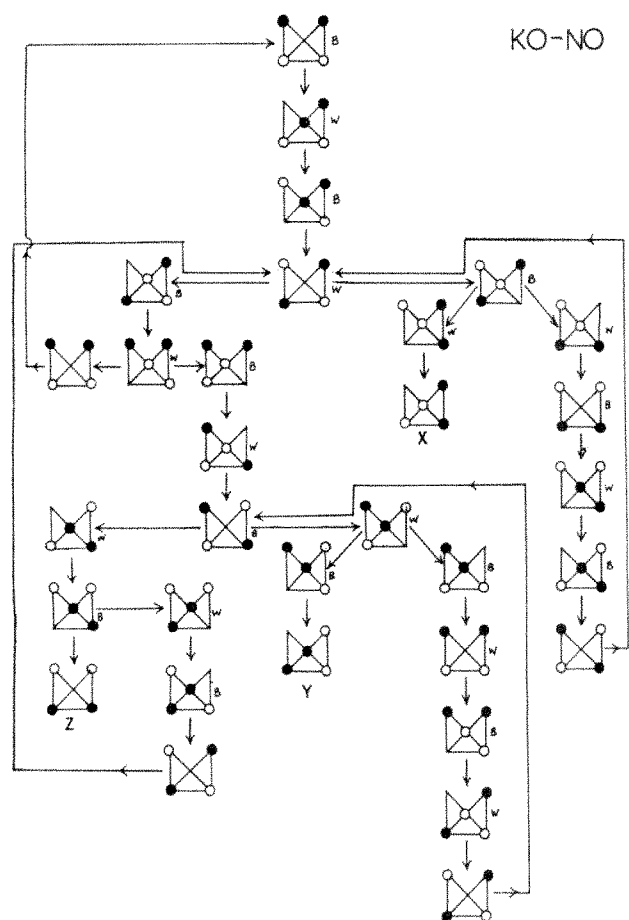
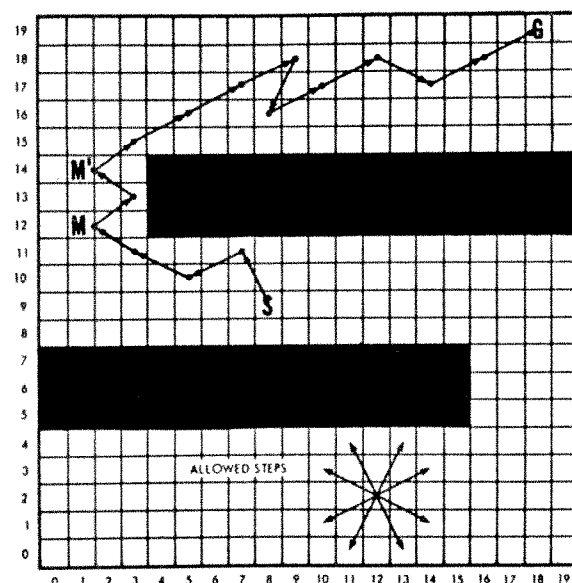


Fig. 2. Half game tree of Ko-No. The position at X is a win for white; that at Y , for black. At Z , the tree repeats with the moves reversed.

Travis was primarily interested in machine as opposed to human intelligence, and he specifically tackled two simple calculi in which the operations are equivalent to the knight's move and a half-knight's move from chess. He used a subset of boards giving domains equivalent to a Go (Wei Chi) board with increasingly complex proscribed areas (Fig. 3). Toda in his Fungus-Eater game used a science fiction terminology and defined the restrictions on the transformations in terms of avarice—uranium greed—and hunger—fungus need. In spite of this deliberate dressing-up, the game is a stylized search or route-finding task, capable of systematic elaboration. Toda himself, "just because they are easy to deal with", deals primarily with versions of his game which in his terminology are discrete, binary, singular and exclusive. With the additional restrictions that the environment is homogeneous, that the game is F -free and that the rewards are situated at the nodes rather than on the pathways, the game becomes isomorphic with the perceptual maze test (Fig. 4).

Travis's tally calculus



Recursive solution of subproblems of Problem 37

The original problem S -to- G was solved by breaking it into the subproblems S -to- M and M -to- G and recursively attacking these. The problem M -to- G was further broken into M -to- M' and M' -to- G before success was achieved.

Fig. 3. Sample problem from Travis: a path must be constructed which goes from start to goal using knight's moves, none of which must finish on the proscribed (black) areas.

Travis's problems, Toda's games and the perceptual maze test are, strictly speaking, puzzles or single-person games, and are each relatively simple elaborations of the basic paradigm A is B . Each is a set of problems in which all the information necessary for a successful solution is available to the problem solver.

Research with the perceptual maze test has shown, in relation to the study of human intelligence, some of the advantages of choosing a set of problems which can be analysed and simulated simply by computer¹¹. Theoretically it would seem likely that the study of artificial intelligence and the development of computer programs which can reason might be expedited, at least initially, by choosing problems which are trivial for man. As

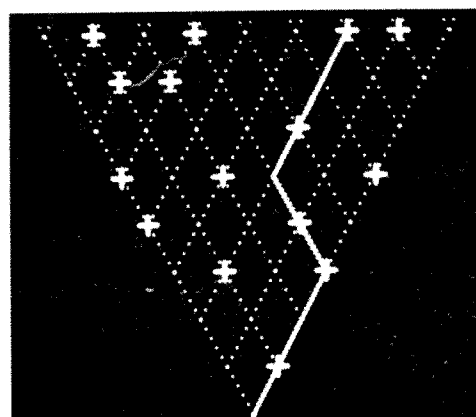


Fig. 4. A sample item from the computer version of the perceptual maze test, together with a solution pathway. The subject's task is to find a pathway from the bottom to the top, passing through a maximum number of target dots.

A. E. and Jagoe have argued⁷, however, research on artificial intelligence and on human reasoning might both be synergetically facilitated by the development of families of problems which are suitable for both areas of research. Using the same terminology as in Table 1, Table 2 lists the parameters of some such problems which have been generated specifically for research on thinking. Some of these, including those mentioned earlier, are puzzles, others are games, the latter differing from the

game and the perceptual maze test, the path selected is determined by the rewards collected and the dangers avoided. In reduced Halma, a set of problems and games of variable complexities is developed from the puzzle paradigm *A is B*, by increasing the number of pieces, by the addition of the hop move and by the introduction of an alien intelligence as an opponent. These changes produce a combination of restrictions which places a premium on the development of patterns which maximize the possibility

Table 2. PARAMETERS OF SOME PROBLEMS GENERATED FOR RESEARCH

Parameter	Fungus-eating game	Tally calculus	Perceptual maze	<i>A is B</i>	Cain and Abel	Reduced Halma
Aim	Capture of pieces	Escape	Capture of pieces	Escape	Race	Alignment (race)
*Complications of board	Limited foresight	Proscribed areas	—	—	—	—
Type of piece	3	1	2	1	2	2
Types of move	Binary	Knights Half-knights	Binary	Variable	Variable	Single step and hop
Alien intelligence	—	—	—	—	+	+

* In all these problems except the Tally calculus the universe, or board, and the number of pieces are the principal experimental variables. With the Tally calculus the variables are the characteristics of the board and the rules governing the moves.

former only in that alternate transformations—"moves"—are determined by a different or alien intelligence. The fact that puzzles and games can be systematically related is important, because it is invaluable to have problems which can be presented in both puzzle and game form. In addition to the Fungus-Eater game, Travis's Tally calculus and the perceptual maze test, Table 2 lists the puzzle paradigm, *A is B*, referred to above, and a basic game, Cain and Abel, together with a more complex game—reduced Halma—in which the elements of *A is B* have been elaborated sufficiently to provide sets of puzzles and games which are challenging and interesting to a wide range of human subjects and which are yet sufficiently

of advance of one organism or army and at the same time minimize that of the other.

This development of reduced Halma from the game paradigm Cain and Abel and hence from the basic problem *A is B* is illustrated in Fig. 5. Cain and Abel is the simplest possible game, namely a competitive puzzle. Cain must go from A to B, Abel from A' to B'. The victor is the first to reach base. In the left-hand diagram there are two armies which have this basic race problem of achieving a defined transformation in the smallest number of operations. If the permissible moves are merely a unitary change in either or both of the *x* and *y* coordinates—a king's move in chess—the problem remains trivial regard-

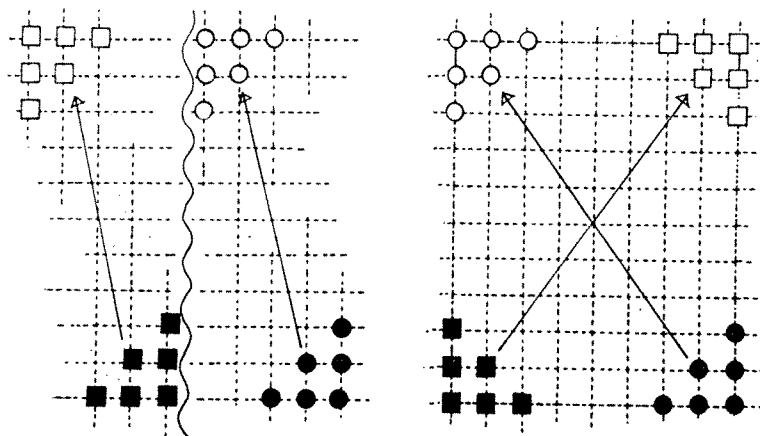


Fig. 5. Games and problems of movement; the organization of movement on a cartesian coordinate system. The left-hand diagram illustrates a simple problem which can be a race or competitive puzzle, that on the right an "interactive" race with the opponents' tracks overlapping.

simple to allow the development of computer programs of a fairly general sort.

An important subgoal of all these problems is the development of optimal patterns of movement. How this can be achieved is determined partly by the rules which determine the types of move or transformation which are permissible, partly by the complexities of the terrain, and partly by the rules which determine the interaction between the men. In the Tally calculus, the Fungus-Eater

less of the size of the armies or their distance from goal. If the "hop" move of Halma is introduced, the problem soon becomes difficult for both man and machine. (The hop is a move which permits a piece, occupying a square next to another piece with a vacant square on the opposite side, to hop into that vacant square: such a hop may be repeated within the same move in any direction as long as there are men to hop over and squares to hop into.) This is because although there is a simple algorithm

illustrated in Fig. 6 which maximizes the rate of movement for an army of any size across an open field, the problems of mobilization at the beginning and "bedding down" at the end rapidly become increasingly complex as army size increases. Further complexity comes from adding boundary restrictions. Complexity of a different order, however, is added by allowing the paths of the two armies to overlap. This "interaction" complexity probably becomes maximal when, as in Halma, the task is what Murray⁵ and Bell⁶ call "replacement"; namely, each army is required to occupy the starting base of its opponent. Fig. 7 shows the symmetrical replacement game presented in puzzle form by Martin Gardner¹⁴. The black pieces must be moved using the minimum number of Halma moves to the positions indicated by the white circles. Fig. 8 shows how for reduced Halma the two principal variables—board size and number of men—can be manipulated experimentally without qualitatively changing the basic concepts of the game. Played on a chess board with ten men a side, reduced Halma is known as Grasshopper.

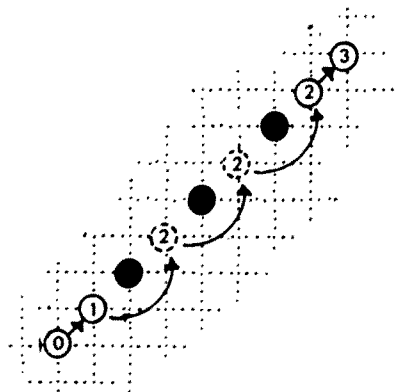


Fig. 6. A simple algorithm defining a "bootstrap" ladder which, using Halma moves, maximizes the rate of movement of n pieces on an unbounded board.

Interaction Complexity

Increasing the number of parameters involved is clearly one way of increasing the complexity of a problem domain. Increasing the number of levels which an existing parameter can take is another. The degree to which parameters interact is a third, and one of the most important sources of complexity. Thus in Go, Halma and chess the board size limits the number of pieces. In Nim and the Mancala games, where stacking is permitted, the two are independent. A different type of interaction cited earlier is that which occurs when an overlap of the fields of operations complicates a competitive race. Another example of an interaction, which can produce an exponential increase in complexity, are the rules which define the "social" relationship between the pieces. Examples are the rule of capture in Go and the hop move in Halma. This is not just a permissive rule, that is to say a convenient method of ignoring an intervening obstruction, but a rule which allows a cooperative or creative relationship to develop between the pieces. In order to find the best move it becomes necessary not only to consider the relationship between all the pieces, but to control the development of these relationships so that the advantage of the position is ultimately divided equally between each piece. Victory does not go to any one individual soldier such as a general or a commander-in-chief, but to the army as a whole. Halma is therefore a political game in which each member of the body politic shares a common intelligence. It is obvious that it is possible to create a true political game by defining situations in which each

man or group of men forming armies, although controlled by different intelligences with restricted methods of communication, have to a varying degree a common aim. Three and four-handed Halma are true political games.

Puzzles and Games: Social Intelligence

The fact that it is possible to simplify the parameters concerned so that they become manageable, yet retain sufficient complexity to make the game interesting, means that it is possible to take effective advantage of the fundamental difference between puzzles and games. In his popular introduction, Abrahams¹⁹ writes that the compleat chess player should play the board rather than the man. But chess is a game and not a puzzle. It is a game because the problem contains "indeterminate" features which are indeterminate not because of chance or random components but because they are determined by the "unknown" structure of an irrational opponent. Most games theorists postulate an inappropriate model, and games theory as it is generally understood relates to the behaviour of fully rational players. Given fully rational proponents, the prisoner's dilemma which has exercised so many psychologists can be seen to be no dilemma for the mathematician. For the laboratory psychologist, however, it is essentially a non-problem because the outcome is a function not of controllable variables but of the estimates each proponent makes of his opponent's value judgments. Such value judgments are necessarily a function of a real rather than a laboratory situation.

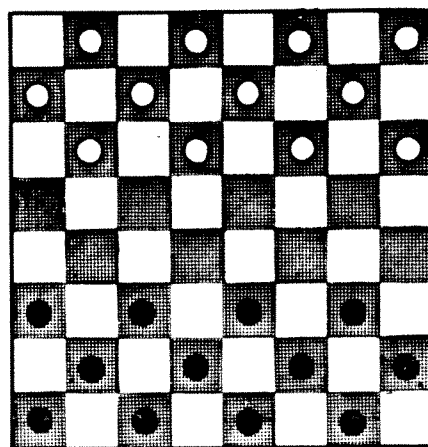


Fig. 7. "Solitaire Halma": the black pieces must be moved using the minimum number of Halma moves (twenty) to the positions indicated by the white circles.

Life is a puzzle only to philosophers. To the poet it is "all a chequer board of nights and days where destiny with men for pieces plays". To the man in the street, it is not a single contest with an omnipotent fully rational opponent but, in the sense proposed by Cedric Smith⁸, a compound game or chequer board of subgames. These require cooperation with, as well as opposition to, other irrational players. In real life, success, whatever the goals or values, goes to the player who can do well in a number of simultaneous games. Hence, ordinary principles of economy suggest that the man who plays the player and who understands the principles and weaknesses of his opponent's strategies will be at a considerable advantage as compared with the player who plays the board. Indeed, many psychologists see survival in a social setting as requiring social intelligence. By this they in fact mean little more than the ability to use cognitive skills to perceive and understand the strategies and skills which

other players bring to cooperative or competitive problem solving.

Programming a computer to play games is a convenient way of modelling a set of strategies in a dynamic form. The success with which a subject competes against such a program will depend on his skill in developing, testing and using to his own advantage theories about the strength and weaknesses of his opponent. The moves that he makes will reflect his ability to analyse his opponents in terms of the general principles which determine their action and will provide a good measure of his powers of abstraction.

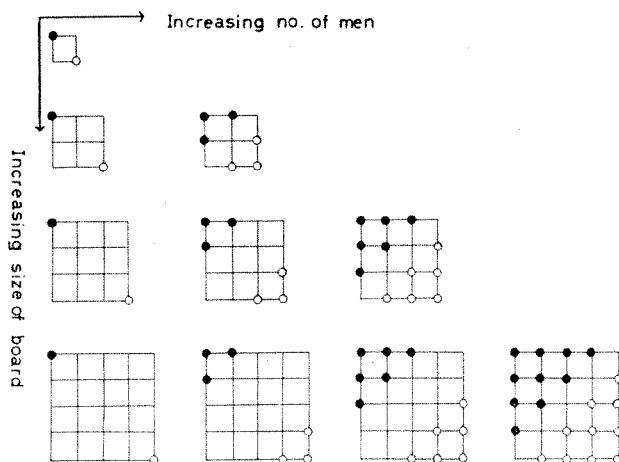


Fig. 8. Various boards for Halma-type games showing how the board size and number of pieces can be independently varied.

To demonstrate the validity of some of these points, one of us (A. T.) has written a program to play Halma-type games. This program is conceptually very simple. It takes no account of its opponent's possible reply and it does not generate any sub-goals, such as simple ladder sequences, like that illustrated in Fig. 6. In fact, it has no look ahead. At each play it merely searches the games tree at the first level and chooses that one of the permissible moves which produces the greatest advance. As programmed at the moment, it is, however, not completely conceptually pure. That is to say, it does not consist only of the implementation of a single calculus but includes a few heuristics derived from practical experience in playing Halma. These have been added, first, to resolve ambiguities, and, second, to give an end game which is adequate to challenge competent human players. With further development, they will be discarded. A laggards routine prevents pieces from dropping too far behind the main army and one or two *ad hoc* subroutines facilitate the bedding down or packing into the goal camp, which constitutes the end game. Including these routines, the present program consists, however, of only 1,400 instructions in the assembly code of the PDP 5/8/12 series of computers. Including the oscilloscope display and input and output routine, the whole program occupies about two-thirds of the 4K store and gives almost instantaneous responses.

Why is such a simple program of interest? How can it help us to evaluate and understand the human problem solver? First, the fact that the program can find all possible legal moves at the first level of search means that it can throw light on the characteristics of good moves which a player overlooks⁷. Second, the program demonstrates very clearly the relative difference in effectiveness of machine execution and human execution of the same strategy. With the smallest Halma games it is possible for human subjects to consider every possible move. As

the size or complexity of the game is increased, human subjects soon become unable effectively to carry out a complete search and not every move is considered, but such moves as are considered are still considered in depth. Increasing the complexity of the game in different ways will affect the mechanical and human problem solver differently. Thus holding board size constant and increasing the number of pieces shifts the balance in favour of the machine. Holding the number of pieces constant and increasing the board size greatly shifts the balance in favour of the human player. At the present stage of development of this program, these findings are relatively trivial but one of the chief advantages of a single game situation such as reduced Halma is that it allows the development of programs of this type which, although logically pure, are sufficiently simple to allow effective development in a number of directions. Thus it is quite practicable with a small Halma game to contrast the effectiveness of trading depth of analysis against completeness of analysis. The problem of selecting which moves or which decisions are those most worthy of exploration at greater depth is of course the critical one for games playing and problem solving in general. With this type of problem, it is possible to program and to contrast strategies which reflect how human problem solvers tackle different aspects of the task with those which reflect an optimal use of machine capacities. This potential for developing families of programs with different characteristics (or strategy sets) and with different abilities is the aspect of this type of game situation which will greatly facilitate the study of social intelligence.

Experience with the very simple program already developed has already highlighted the great advantage which the human player gains when he understands the strategies which are at the disposal of his computer opponent. Indeed, our rather limited experience to date has underlined the difficulty that even highly intelligent human subjects have in setting up reasonable hypotheses about the abilities or strategy sets of an opponent with an alien intelligence.

Although formal experiments along the above lines remain to be carried out, we would argue that not only is this approach intuitively attractive but also that the value of developing problems which are amenable to solution by systematically derived machine programs has been demonstrated by research already undertaken with the perceptual maze test¹¹. While the problems are more complex with reduced Halma, they are not, as with chess, outside the reach of computer techniques which are either currently available or which are likely to be available in the near future.

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Is "I-DNA" derived from Nuclear DNA?

by

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On the basis of double radioactive labelling and buoyant density studies, it is concluded that "I-DNA" is not a separate entity from nuclear DNA but may be an artefact derived from it.

RECENTLY, Bell¹ described "a special class of cytoplasmic DNA", called informational DNA, or "I-DNA" occurring in eukaryotic cells. It was said to be synthesized with logarithmic kinetics, by contrast with nuclear DNA, which is formed at a linear rate. Fromson *et al.*² also found fractions of "I-DNA", but the yield strongly depended on the technique used for cell disruption, and it was therefore concluded that the material was nothing more than contaminating nuclear DNA.

We have tried to characterize the "I-DNA" fraction by comparing its properties with those of nuclear DNA. The studies were performed with mouse lymphoma cells L_{5178Y} (ref. 3), growing in suspension in Fischer's medium supplemented with 10 per cent horse serum.

Sedimentation Characteristics

The banding properties of "I-DNA" in sucrose were found to be nearly the same as described by Bell¹. After incubation with ³H-thymidine we found a DNA peak, sedimenting at 8S (Fig. 1). We could not collect this DNA fraction by precipitation with cold trichloroacetic acid on 'Millipore' filters, but using cetyltrimethylammonium bromide precipitation⁴ it was possible to detect small quantities of "cytoplasmic" DNA.

The banding profiles of nuclear DNA and "I-DNA" in CsCl are shown in Fig. 2. Both materials have the same average buoyant density of 1.701. The banding pattern of nuclear DNA includes a minor component with an average buoyant density of 1.709. The banding profiles

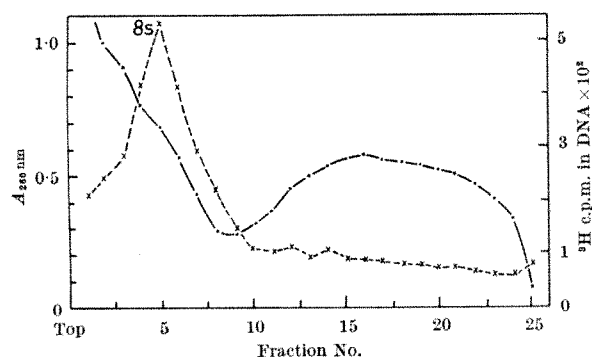


Fig. 1. Preparation of "I-DNA" in a sucrose gradient. 2.4×10^6 L_{5178Y} cells (1.2×10^4 cells/ml.) were incubated for 4 h at 37° C with 0.1 mCi/ml. [*methyl-³H*] thymidine (specific activity 23.5 Ci/mmol; Radiochemical Centre, Amersham); the cells were disrupted by homogenizing at 0° C in a Potter-Elvehjem homogenizer (1,000 r.p.m. fifteen times up and down). Nuclei were removed by centrifugation at 1,000g for 5 min in a Sorvall 'RC2-B' ('HB-4' rotor). 1.0 ml. of the supernatant suspension was layered on a linear gradient of 15–30 per cent sucrose, buffered with 1.5 mM MgCl₂, 3 mM CaCl₂, 10 mM Tris, 10 mM NaCl (pH 8.0) and centrifuged at 40,000 r.p.m. in an 'SW 40' rotor (Spinco 'L2-50B') at 4° C for 4 h. Twenty-five fractions were collected, analysed for ultraviolet absorbance (A_{260}), —, and precipitated with cetyltrimethylammonium bromide⁴. Precipitates were put on 'Millipore' filters ('HA' 0.45 μ m) and washed with distilled water. Dried disks were transferred to counting vials, solubilized in 1 ml. methoxyethanol, immersed in 10 ml. of dioxane scintillator (100 g naphthalene, 4 g PPO and 0.3 g POPOP in 1 l.) and counted on a Packard scintillation spectrometer model 3380, \times — \times .

of the two DNAs are distinguishable by the width at their bases: for nuclear DNA the profile has a narrow base extending between densities of 1.67 to 1.76; for "I-DNA" the base is wider from 1.62 to 1.77.

Kinetics of Synthesis

The kinetics of synthesis of "I-DNA" and nuclear DNA were measured with the double labelling technique. The plots of radioactivity and absorbance in the DNA

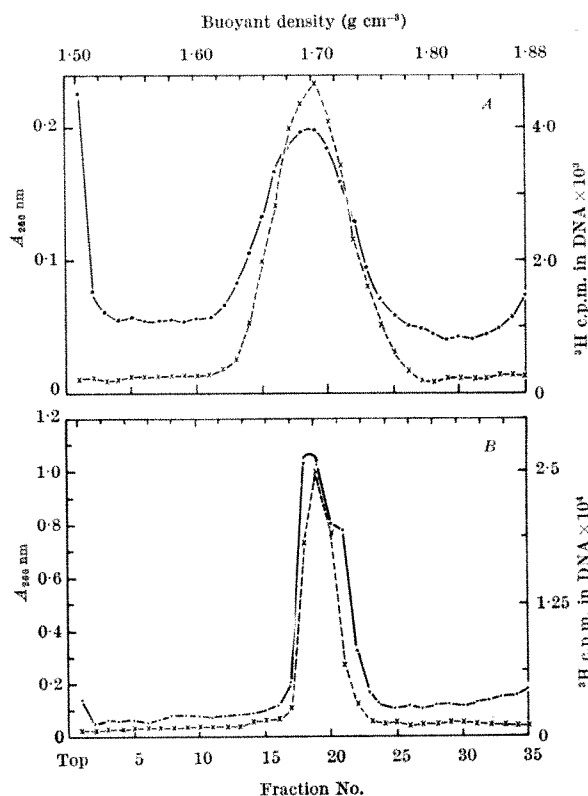


Fig. 2. Buoyant densities of labelled "I-DNA" and nuclear DNA. 1.4×10^7 L_{5178Y} cells (1.3×10^4 cells/ml.) were incubated for 2 h at 37° C with 10 μ Ci/ml. ²¹⁴C-thymidine (specific activity 52.8 mCi/mmol; New England Nuclear Corp., Boston), followed by addition of 0.1 mCi/ml. [*methyl-³H*] thymidine (specific activity 23.5 Ci/mmol) for 2 h at 37° C. Details for the separation of cytoplasmic extract from nuclei were as in Fig. 1. The materials, in CsCl solution buffered with 15 mM Tris (pH 8.0) (initial density: 1.71 g/cm³) were centrifuged at 33,000 r.p.m. in an 'SW 40' rotor at 20° C for 72 h. Thirty-five fractions were collected. An aliquot of 20 μ l. of each fraction was applied to a filter disk (Whatman No. 1) and treated further according to the Bollum technique⁵. The disks were put in 10 ml. dioxane scintillation solution and radioactivity was counted. A, 10 ml. cytoplasmic extract containing the "I-DNA" fraction was freed from the bulk protein and RNA by CsCl centrifugation; the radioactive fractions ("I-DNA") were collected and re-centrifuged. Their banding UV profile, —, and the measured distribution of the radioactivity, \times — \times , are shown in the graph. B, The nuclear DNA was prepared⁶ and dissolved in CsCl solution. The results after centrifugation are shown.

region are congruent (Fig. 2) (maximum for nuclear DNA is at an average buoyant density of 1.703 compared with 1.699 for "I-DNA"). Protein and RNA components are free of radioactivity. The average values, with standard deviations, of the channel ratio $^3\text{H}/^{14}\text{C}$ of the active fractions are 27.75 ± 1.39 and 25.96 ± 4.06 , for nuclear DNA and for "I-DNA" respectively (two samples counted per fraction; nuclear DNA was in fractions 17–22, "I-DNA" in 14–24). There is no significant difference between the two values (Student *t* test; $P > 0.2$). The specific activity of the tritiated nuclear DNA (55 nCi/0.1 mg) was nearly identical with that of the "I-DNA" (60 nCi/0.1 mg).

Conclusion

We could find no significant evidence for the existence of "I-DNA". The preparation which we have sought to resolve has the same buoyant density, the same rate of synthesis (by contrast with the results of Bell¹) and the

same specific radioactivity as nuclear DNA. The base of the banding profile for "I-DNA" in a CsCl gradient is wider than that for the nuclear DNA, which may indicate that "I-DNA" largely consists of short pieces formed during homogenization of the cells. We conclude that it is an artefact in our eukaryotic cell system derived from nuclear DNA. Fromson *et al.*² interpret the association of RNA with cytoplasmic DNA described by Bell¹ in terms of a co-sedimentation effect without any connexion of structural relevance.

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Formation of a Ternary Complex between Formylatable Yeast Met-tRNA, GTP and Binding Factor T of Yeast and of *E. coli*

by

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Both formylatable and non-formylatable met-tRNAs of yeast bind to the elongation factor T which transfers aminoacyl-tRNA to the ribosome. This means that yeast cannot use the same mechanism as *E. coli* for distinguishing initiator tRNA from other aminoacyl tRNAs.

CASKEY *et al.*¹ demonstrated that about 40–50 per cent of the methionine tRNA of liver can be formylated by incubation with the *Escherichia coli* transformylase, even though there is no transformylase in the cytoplasm. Similar results were obtained with yeast Met-tRNA^{2,3}. These eukaryotic formylatable and non-formylatable Met-tRNAs have been separated^{1–4}, and the formylatable fraction was found to share with the bacterial chain-initiating Met-tRNA^{Met} the property of not being accepted by internal AUG^{2,3,5}. Recently, Smith and Marcker⁶, and Brown and Smith⁷, suggested that the formylatable eukaryote tRNA^{Met} (designated by them as tRNA_i^{Met}) is an initiator tRNA on eukaryote cytoplasmic ribosomes. Bacterial Met-tRNA^{Met}, by contrast with other aminoacyl-tRNAs, does not form a ternary complex with *E. coli* supernatant transfer factor T (T_u-T_s) and GTP⁸. The unique rejection by T of bacterial Met-tRNA^{Met} has been related to its initiator function⁶. Although it now seems well established⁹ that it is the T_u component that ultimately forms the ternary complex, we refer here to T as binding factor because the T_u-T_s complex was used in all our experiments.

In view of recent indications that unformylated Met-tRNA^{Met} may act as initiator in the eukaryote cytoplasm, we have tested its interaction with the binding factor T of yeast as well as of *E. coli*. We find the most reliable method for quantitation of binding capacities is the 'Sephadex G-50' filtration method described by Gordon¹⁰. He observed¹¹ that, in the ternary complex thus obtained, equimolar amounts of GTP and aminoacyl-tRNA are bound to the T factor; similar results were reported by Ravel *et al.*¹². Recently, one of us (D. R.) confirmed¹³ the stoichiometry between ^3H -GTP and ^{14}C -Phe-tRNA using binding to yeast T factor and 'Sephadex G-200' for filtration; in this way it was shown that yeast binding factor T forms a ternary complex similar to bacterial T.

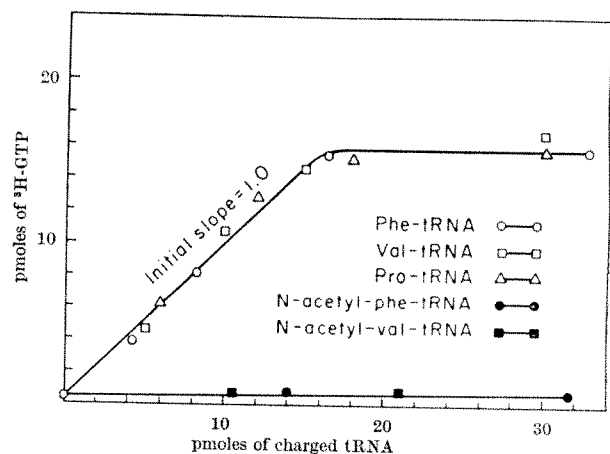


Fig. 1. Formation of the ternary complex with *E. coli* factor T and ^3H -GTP, and either ^{14}C -Pro-tRNA, ^{14}C -Phe-tRNA, ^{14}C -Val-tRNA, or N-acetylated ^{14}C -aminoacyl-tRNA. *E. coli* factor T was isolated up to the hydroxylapatite step as described by Gordon¹⁰. This preparation was 70 per cent pure. Isolation of the aminoacyl-tRNA synthetases from *E. coli* and esterification of the amino-acids with *E. coli* tRNA (General Biochemicals, Inc) were carried out by the method of Muench and Berg¹³. The specific activities of the ^{14}C -amino-acids (Schwarz BioResearch) ranged from 75–450 mCi/mmol. N-Acetyl- ^{14}C -Phe-tRNA and N-acetyl- ^{14}C -Val-tRNA were from *E. coli*, and were prepared and assayed for acetylation by the method of Haenni and Chapeville¹⁴. In one experiment, uncharged tRNA was treated with acetic anhydride and re-isolated to compare the binding to T factor of pretreated tRNA, charged with phenylalanine or valine, with the binding of similarly charged untreated tRNA. The ternary complex was formed with 18 μg of *E. coli* T factor, 280 pmoles of ^3H -GTP (specific activity 6.05 Ci/mmol; New England Nuclear), and various amounts of ^{14}C -aminoacyl-tRNA, in a total reaction volume of 100 μl , which contained 100 mM NH₄Cl, 60 mM KCl, 10 mM magnesium acetate, and 20 mM Tris-HCl (pH 7.4). The complex was isolated by passing the reaction mixture through a 'Sephadex G-50' column (1.2 \times 23 cm). Each plot in the figure represents a gel filtration run with the indicated amount of aminoacyl-tRNA. In view of the exact stoichiometry obtained recently with similar methodology^{11–13}, only 'Sephadex-excluded' ^3H -GTP was assayed for in all experiments shown in this and the following figures.

Formation of the Ternary Complex

In all experiments reported here, formation of the complex was assayed by adding increasing amounts of ^{14}C -aminoacyl-tRNA to an excess of binding factor and tritiated GTP. After filtering the mixture through 'Sephadex G-50', the excluded radioactivity of ^3H -GTP was compared with the amount of aminoacyl-tRNA added. Only the ^3H that appeared in the void volume of the column was measured. We felt this was sufficient, for yeast and bacterial T factors had been demonstrated to bind GTP and aminoacyl-tRNA in equimolar amounts with a methodology similar to that used here¹¹⁻¹³. In our conditions, ^3H -GTP was bound neither to the yeast nor to the bacterial T factors in the presence of uncharged tRNA.

Largely in order to check the method of analysis, it is shown in Fig. 1 that stoichiometric amounts of GTP were bound to the T factor when increasing concentrations of Phe, Val and Pro-tRNA were added to an excess of ^3H -GTP and *E. coli* T. This is indicated by a slope of 1.0 up to the saturation point when no further binding occurs; for a check of generality, Thr, Leu and Gly-tRNAs were tested with analogous results (not shown). Although in proline there is a substitution of the amide nitrogen, this does not alter the binding capacity of Pro-tRNA. By contrast, we confirm (lower curve, Fig. 1) that N-acetylation prevents binding.

In this experiment, the tRNA preparations used for charging with phenylalanine and valine were pretreated with acetic anhydride to meet the objection that in the method of Haenni and Chapeville¹⁶ the anhydride may cause acetylation of tRNA and thereby inhibit binding.

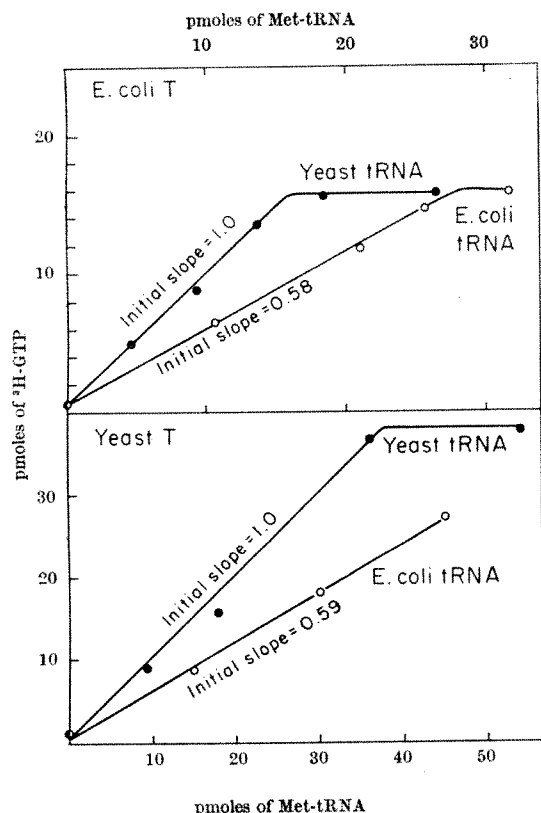


Fig. 2. Formation of the ternary complex with yeast and *E. coli* binding factors T, ^3H -GTP, and ^{14}C -Met-tRNA from homologous and heterologous sources. tRNA from yeast (Miles Laboratories) was charged with ^{14}C -methionine (specific activity 110 $\mu\text{Ci}/\mu\text{mole}$) by a homologous enzyme preparation as described¹⁵. For *E. coli* ^{14}C -Met-tRNA, see ref. 15. The binding factor T from yeast was isolated as previously described¹³; this preparation was 50–60 per cent pure as estimated by gel electrophoresis (unpublished data of D. R.). Complex formation with the yeast or *E. coli* binding factor was carried out as described in Fig. 1, except that with the yeast factor 280 μg of protein, 560 pmoles of ^3H -GTP, and ^{14}C -Met-tRNA were used as indicated.

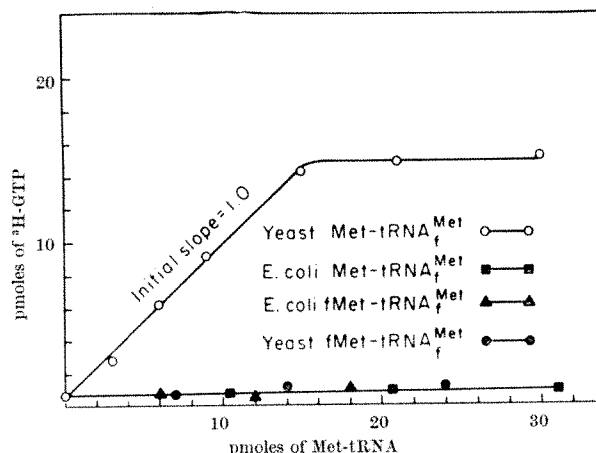


Fig. 3. Formation of the ternary complex with the *E. coli* binding factor T, ^3H -GTP, and the formylated or non-formylated ^{14}C -Met-tRNA_{Met} from yeast or from *E. coli*. Formylation of the ^{14}C -Met-tRNA_{Met} from *E. coli* was carried out as described¹⁸. Yeast Met-tRNA_{Met} was prepared with 44 mg of commercial yeast tRNA (Miles Laboratories), purified yeast synthetases¹⁹, and ^{14}C -methionine (specific activity 110 $\mu\text{Ci}/\mu\text{mole}$). This ^{14}C -Met-tRNA preparation (specific activity 20.6 pmoles of methionine per A_{260} unit) was phenoxyl-acetylated¹⁹ and chromatographed on benzoylated DEAE-cellulose at 4°C (ref. 17). (BD-cellulose from Schwarz BioResearch.) Phenoxyl-acetyl [^{14}C]-Met-tRNA was eluted from the column (1.2 \times 14 cm) with a linear gradient of ethanol (0–20 per cent ethanol in 0.8 M NaCl—10 mM MgCl_2 —10 mM acetic acid, pH 4.5; each mixing chamber contained 50 ml). Two ^{14}C -Met-tRNA peaks were obtained, one at about 5 per cent ethanol, the other at 20 per cent ethanol. The N-substituted ^{14}C -methionyl group was stripped from the tRNA¹⁹, and the tRNA was recharged with ^{14}C -methionine (specific activity as already given). The two separated ^{14}C -Met-tRNA species from yeast were assayed for their formylability with the *E. coli* transformylase and folinic acid. The extent of formylation was estimated by paper electrophoresis¹⁸. The ^{14}C -Met-tRNA species obtained at 5 per cent ethanol from the column contained less than 10 per cent formylatable ^{14}C -Met-tRNA and is therefore referred to as ^{14}C -Met-tRNA_{Met}. The other ^{14}C -Met-tRNA species (eluted at 20 per cent ethanol) represented a mixture consisting of 40 per cent formylatable and 60 per cent unformylatable Met-tRNA. This mixture was further purified by repeating the BD-cellulose chromatography. For this purpose, it was first formylated with the *E. coli* transformylase, and then the non-formylatable part was phenoxylacetylated as described above. It was finally rechromatographed on a BD-cellulose column as outlined above. The formylated yeast ^{14}C -Met-tRNA_{Met} was eluted with 0.8 M NaCl—10 mM acetic acid (pH 4.5)—10 mM MgCl_2 , and then contained only about 10 per cent non-formylatable Met-tRNA (specific activity 1,250 pmoles of methionine per A_{260} unit). For the experiments with unformylated yeast ^{14}C -Met-tRNA_{Met}, the formylmethionyl group had to be stripped from the tRNA_{Met} and recharged with ^{14}C -methionine.

The results of Fig. 1 demonstrate that pretreatment had not altered the reactivity of aminoacyl-tRNA with T, and the failure of these N-acetylaminoacyl-tRNAs to bind must be attributed to the blocking of the amino group.

Binding of Met-tRNAs with T Factors

Non-fractionated *E. coli* and yeast tRNAs, charged with methionine, were first tested in a manner similar to that described. When the mixture of *E. coli* Met-tRNAs was tested (Fig. 2), the slope of the straight line up to saturation corresponding to the ratio of GTP and Met-tRNA was 0.58–0.59 rather than 1.0, indicating that only about 60 per cent of the *E. coli* Met-tRNA was bound to T factors of either *E. coli* or yeast. This shows that only part, presumably the non-formylatable portion of the *E. coli* Met-tRNA, was bound to T factor; the rest, about 40 per cent of the Met-tRNA, did not react in the binding test. When the unfractionated yeast-derived mixture was tested, however, the slope was 1.0 up to saturation, indicating stoichiometry between GTP and the mixture of yeast Met-tRNAs. Because this yeast Met-tRNA contains formylatable and non-formylatable Met-tRNA in approximately equal amounts^{2,3}, both species seem to react equally well with either the yeast (Fig. 2, lower section) or the *E. coli* T factors (Fig. 2, upper section).

To prove more convincingly the binding of formylatable Met-tRNAs of yeast to the T factors, it was desirable to

carry out tests with reasonably pure tRNA fractions. The separation of the yeast Met-tRNAs was done as outlined by Henes *et al.*¹⁷ for *E. coli* Met-tRNA. This method fractionates the phenoxy-acetylated methionyl-tRNAs from yeast on BD-cellulose at 4° C. One fraction only of the two species was formylatable with the *E. coli* transformylase and folinic acid (see legend to Fig. 3). The isolated non-formylatable Met-tRNA^{Met} and formylatable Met-tRNA^{Met} from yeast were assayed for their binding capacity. The experiments presented in Fig. 3 confirm our conclusion that formylatable yeast Met-tRNA is not rejected by the T factors, insofar as yeast Met-tRNA^{Met} is bound as a ternary complex in stoichiometric amounts to GTP. The fact that the preparation of Met-tRNA^{Met} was 90 per cent formylatable and still contained 10 per cent tRNA^{Met} does not affect this conclusion for, if it were rejected and only Met-tRNA^{Met} was bound, the expected slope would be 0.1. The slope of 1.0 found experimentally just confirms that both formylatable and non-formylatable factors form a ternary complex, as might be expected from the results of Fig. 2 where the natural mixture of both shows a slope of 1.0 by contrast with a slope of 0.6 with the mixture of *E. coli* Met-tRNAs. In other experiments (not included) the purified Met-tRNA^{Met} of yeast was directly tested for ternary complex formation and exhibited a slope of 1.0. Blocking the amino group of the yeast Met-tRNA^{Met} by formylation, however, caused rejection by the T factor (Fig. 3). Similarly it was found (not shown) that acetylation of the amino group of yeast Met-tRNA^{Met} by the method of Haenni and Chapeville¹⁶ prevents formation of a ternary complex with yeast T factor. On the other hand, for pure Met-tRNA^{Met} of *E. coli*, we confirm that it is not bound to T factor. This indicates that in Fig. 2 the 40 per cent of *E. coli* tRNA charged with methionine that did not bind was indeed the Met-tRNA^{Met}.

Although the recent observations by Smith and Marcker⁶ suggest that the formylatable form of the cytoplasmic eukaryote Met-tRNA may indeed be the initiating tRNA for eukaryotic protein synthesis, our experiments show both formylatable and non-formylatable cytoplasmic Met-tRNAs of yeast to react equally well with our T fractions.

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Detection of Inactive Enzyme Molecules in Ageing Organisms

by

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Using immunochemical techniques, evidence is obtained that older nematodes (*Turbatrix aceti*) contain two populations of enzyme molecules—one active and the other totally inactive.

THE synthesis and accumulation of non-functional protein molecules may play a significant part in senescence and the eventual death of organisms. In this communication we report the use of immunological techniques to look directly for the presence of catalytically altered or inactive enzyme molecules in ageing nematodes. The immunological approach is both specific and sensitive and allows for the expression of results in units of catalytic activity per unit of antigenic activity, thus allowing for the detection of partially or totally inactive enzyme molecules present in the form of cross reacting material (CRM). This approach has been successfully used in detecting cross reacting protein in bacterial and fungal mutants lacking specific enzyme activities arising from single amino-acid changes caused by point mutations¹.

We chose the nematode *Turbatrix aceti* as a model organism, which has proved suitable for research on ageing for the following reasons²: its life span is short—a mean of 25–30 days; it has a fixed number of cells after hatching, with the exception of the reproductive system; it lacks regenerative capacity³; it possesses a relatively small number of cell types and exhibits full differentiation at the time of hatching; and it can be raised in axenic conditions in quantities suitable for biochemical studies⁴.

Previous studies have shown that the specific activities of several enzymes in *T. aceti* show a general decline with age⁵. It was not known, however, whether this decline in activity was the result of a reduction in the number of enzyme molecules per cell or of the progressive formation or accumulation of inactive molecules. The enzyme

chosen for our present studies, isocitrate lyase⁶, also showed a decline in specific activity with age (see below). This enzyme was presumed to be a good immunogen because it is not present in higher organisms and has been reported to have a high molecular weight in both *Chlorella pyrenoidosa*⁷ and *Pseudomonas indigofera*⁸. Moreover, this enzyme has been shown to consist of a single molecular species in certain other experimental systems^{7,8}.

Turbatrix aceti were grown in sterile axenic culture conditions as described by Rothstein and Cook⁴. Populations of *T. aceti* of uniform age were grown and, at various times after the initiation of culture, populations of uniform age were harvested, thoroughly cleaned of medium components, lyophilized and stored at -20°C until used for enzyme analysis².

Lyophilized populations of *T. aceti* of uniform ages were suspended in cold 0.32 M sodium phosphate buffer containing 5 mM EDTA and 0.5 mM dithiothreitol. Each suspension was homogenized at 400 atmospheres in a cold French pressure cell. The homogenate was incubated at 4°C for 10 min with 0.5 per cent 'Sarkosyl' and subsequently centrifuged at 30,000g for 30 min. The pellet was discarded and the supernatant, referred to as crude enzyme, was maintained at 4°C for enzyme assay.

Synchronized populations of *T. aceti* ranging in age from day 0 to day 45 were assayed for isocitrate lyase activity. For most age points, several determinations were made on two or more independently grown populations. The specific activity of isocitrate lyase in *T. aceti* starts at a high level in early life and gradually decreases with increasing age (Fig. 1). This decrease in enzyme activity could either represent a true reflexion of the amount of enzyme molecules present in the ageing organism or be attributed in whole or in part to the production and/or accumulation of molecules with impaired enzymatic function. To distinguish between these two alternatives the following series of experiments were performed. Antibody to the crude enzyme preparation of 5-day-old *T. aceti* was prepared by immunizing a rabbit by repeated intradermal and intramuscular injections of the crude enzyme preparation in emulsion with an equal volume of Freund's complete adjuvant.

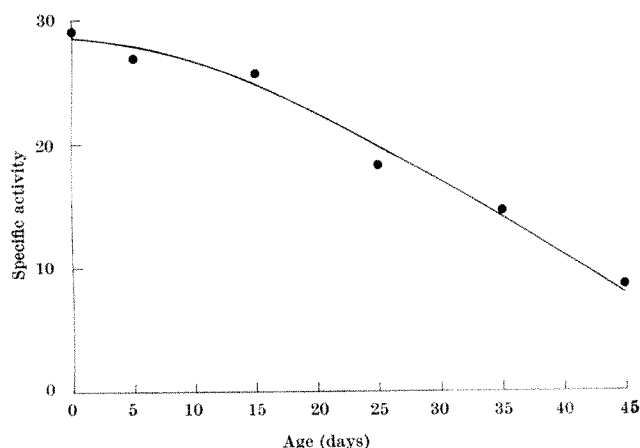


Fig. 1. The specific activity of isocitrate lyase in homogenates of populations of *T. aceti* of various ages. Isocitrate lyase was assayed according to a modification of the method of Dixon and Kornberg⁹. Cold crude enzyme (70 μl .) was added to 0.9 ml. of reaction mixture at 30°C , containing 67 μM sodium phosphate (pH 6.9), 5 μM MgCl_2 , 3.3 μM phenylhydrazine-HCl and 2 μM cysteine-HCl. The enzymatic reaction was initiated by the addition of 25 μl of 0.1 M sodium DL-isocitrate. The formation of glyoxylic acid phenylhydrazone was measured at 324 nm in a double beam spectrophotometer, against the reaction mixture plus crude enzyme without the addition of substrate. Specific activities, expressed as nmoles of glyoxylate formed per mg protein per min, were computed for the enzyme using $E_{324}^{1\%} = 1.7 \times 10^4$ for glyoxylic acid phenylhydrazone. Total protein in the homogenates was determined by the method of Lowry *et al.*¹⁰. Each point represents the average of 7-13 individual experiments.

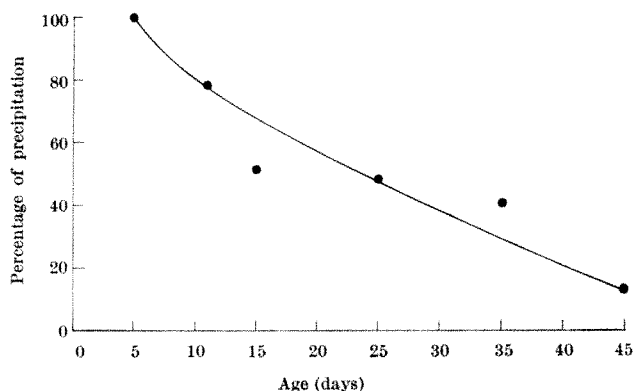


Fig. 2. Precipitation by antibody of isocitrate lyase activity in homogenates of populations of *T. aceti* of various ages. In each experiment all homogenates were adjusted to the same initial activity. Antibody was added to a final concentration of 8.75 per cent. Results are expressed as percentage of the original enzyme activity precipitated. Maximum precipitation observed in the 5 day preparation was designated as 100 per cent. All results are expressed relative to the 5 day preparation. Each point represents the average of 3-8 individual experiments.

Before the commencement of the immunization schedule the rabbit was bled for normal serum.

The effect of antibody on enzyme activity was measured by mixing various amounts of antibody, normal rabbit serum or phosphate buffer containing EDTA and dithiothreitol with crude enzyme preparations of synchronized populations of various ages. At the onset of each individual experiment all crude enzyme preparations were adjusted to the same initial activity. Crude enzyme preparations of each age were divided into three aliquots, one made 8.75 per cent with antiserum, the second made 8.75 per cent with normal rabbit serum and the third a control of equal volume and initial enzyme activity in buffer alone. The amount of antibody added was predetermined to allow all samples to fall within the sharply ascending antigen excess part of the precipitation curve in order to increase the sensitivity of the assay and allow for observation of differences in precipitation of the enzyme in different age samples. It was found that normal rabbit serum had no effect on enzyme activity and gave the same results as the enzyme in buffer controls. The activity of the enzyme was measured immediately after addition of antibody and in certain experiments once again after 1 h, and 24 h incubation at 4°C . It was found that there was a certain degree of inactivation of the enzyme on initial contact with antibody. No such inactivation was noted in the normal rabbit serum controls. Subsequent to this inactivation of initial contact with antibody, the activity of the enzyme remained stable at 4°C . The antibody-homogenate mixtures as well as normal rabbit serum and buffer controls were incubated overnight at 4°C and then centrifuged at 12,000g for 15 min to remove any antigen-antibody precipitate that might have formed. The resulting supernatant was then assayed for residual enzyme activity.

As the populations increase in age, less enzyme is precipitated per fixed amount of antibody (Fig. 2). Thus, for the same level of enzyme activity, crude enzyme preparations from old animals contain more antigenic material than is detected in young animals. Because it has been described in certain other systems^{7,8} and will be demonstrated for this system that isocitrate lyase consists of a single molecular species the results depicted in Fig. 2 may be interpreted as follows: the increase in detectable antigenic material in ageing populations may be attributed to the reduced activity of all the enzyme molecules present or to the presence of some enzyme molecules with a normal degree of activity and others totally lacking enzymatic function.

If the amount of antiserum is increased it is possible to precipitate comparable proportions of enzyme activity

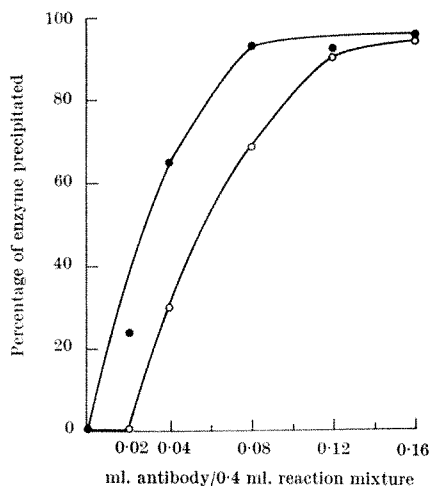


Fig. 3. The precipitation of isocitrate lyase activity from homogenates of 5 and 35 day old populations with increasing amounts of antibody. The initial activities of both preparations were adjusted to be the same. Results are expressed as the percentage of the initial enzyme activity precipitated by antibody. ●, 5 day old; ○, 35 day old.

from populations of 5-day and 35-day-old animals (Fig. 3). The precipitation curves of enzyme from 5 and 35-day-old animals demonstrate the identity of the enzyme in both preparations, for they exhibit the same slope and degree of maximal per cent precipitation¹¹. The 35-day preparation is shifted to the region of higher antibody concentration, indicating the presence of a considerably higher concentration of enzyme antigen per unit of enzymatic activity than is present on day 5.

To establish whether the difference in enzyme activity per unit of antigen may arise from the presence of an inhibitor in older populations, crude enzyme preparations of young and old animals were mixed together in equal volumes and tested immediately after mixing as well as after incubation overnight at 4° C. These experiments failed to demonstrate the presence of an inhibitor of enzyme activity in homogenates of ageing populations.

These experiments indicate either that the isocitrate lyase present in ageing populations of *T. aceti* is composed of enzyme molecules with lower activity than that present in young adults or that the older organisms contain two populations of enzyme molecules, one with completely normal activity—like that of the young adults—and the other, totally inactive molecules which can be detected only as cross-reacting material recognized by antibody. Two lines of evidence make the latter situation the more probable. When the antibody and enzyme interact, some of the enzyme is inactivated, because of the binding of antibody with regions of the enzyme molecule in or very near to the active site of the enzyme¹². If the properties of the area of the active site are the same for all the enzymatically active isocitrate lyase molecules of old as well as young animals, then they ought to be inactivated to the same degree by increasing amounts of antibody. If, on the other hand, the entire population of enzyme molecules bear altered active sites, one ought to see differing inactivation curves when enzyme activity is kept constant and the amount of antibody is varied. In Fig. 4 it is clear that the inactivation of enzyme by increasing amounts of antibody is the same for the enzyme of 5 and 35-day-old animals, thus demonstrating the identity of the active site of the enzyme from old and young populations. The second line of evidence which leads us to conclude that the active enzyme detected in older populations is the same as the enzyme of younger animals comes from studies of the heat inactivation of the enzyme. In Fig. 5 the effect of exposure of crude enzyme preparations of animals 5 and 45 days old to elevated temperature is shown. The inactivation curves are virtually parallel, thus attesting to the identity of

the two active enzyme populations. These results strongly indicate that in old animals the population of enzyme molecules consists of a proportion of fully active molecules and a large amount of altered inactive molecules.

The alteration in enzyme structure resulting in inactive molecules may arise from one of three possible mechanisms or any combination of them: (a) modification of a large proportion of enzyme molecules in the old animal after their synthesis; (b) changes in primary structure caused by errors during the synthesis of enzyme molecules¹³, and (c) progressive accumulation of an inhibitor of enzyme function. Our experiments testing enzyme activity in mixed preparations from young and old animals eliminate the third possibility. The first possibility seems most likely, for random errors in protein synthesis would result in a proportion of partially

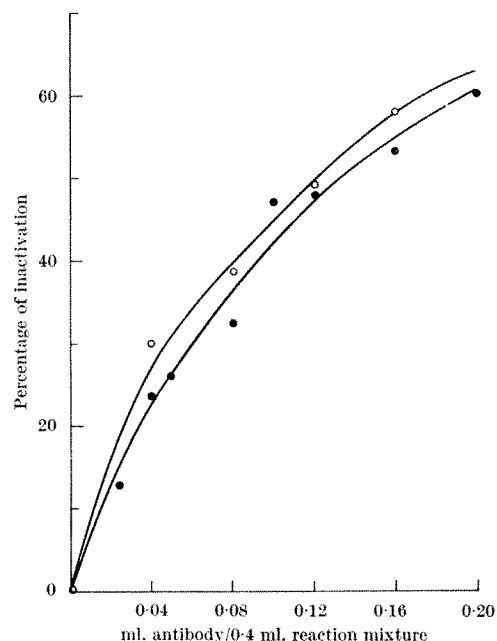


Fig. 4. The inactivation by increasing amounts of antibody of isocitrate lyase activity of homogenates of 5 and 35 day old *T. aceti*. ●, 5 day old; ○, 35 day old.

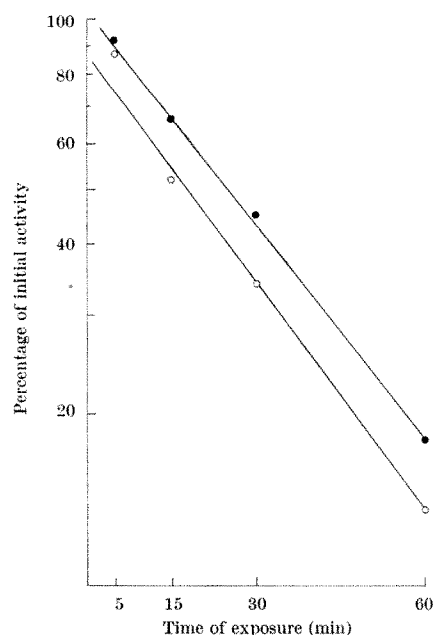


Fig. 5. The inactivation at 45° C of isocitrate lyase activity of homogenates of 5 and 45 day old organisms.

active enzyme molecules; our results demonstrate that the enzyme population is composed of either inactive or fully active molecules for isocitrate lyase. The nature of the chemical modification of the isocitrate lyase molecules which lead to their inactivation is unknown.

It is interesting to note that Palmer and Papaconstantinou¹⁴ have recently reported a post-synthetic chemical modification in a subunit of adult α -crystallin, although this modification does not result in immunological change of the resultant subunit.

Our findings also indicate that investigations of total enzyme activity as a function of age cannot be expressed merely in terms of specific activities. If for a given unit of enzyme activity the old cell synthesizes a considerably larger number of molecules than the young cell, the burden on the aged cellular system in terms of energy expenditures and synthetic capacity is much greater and probably has deleterious consequences. At present, it is not known, however, at what levels of faulty proteins the physiological functions of eukaryotic cells become altered.

The same approach must be repeated with purified enzyme from other cellular systems and other organisms

in order to investigate the universality of the phenomenon reported here. If found to be universal, it should have important implications for the understanding of the mechanisms of ageing.

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X-linked Gene for Testicular Feminization in the Mouse

by

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The condition of testicular feminization in mice resembles that in man and other mammals. It is clearly X-linked. There is no evidence at present that the gene for testicular feminization is involved in mouse spermatogenesis, at least up to the spermatocyte stage.

THE genetic defect of sexual differentiation known as testicular feminization is well known in man^{1,2} and has been described in rats³ and in cattle⁴. Affected human individuals have an XY chromosome complement but the external genitalia develop as in females, so that the sex is classified at birth as female. Internally there are small testes, but no other reproductive organs, either male or female. It is thought that the testes produce androgens normally, but the target tissues, which would normally be induced by these androgens to develop in a masculine manner, fail to respond^{5,6}. Bardin *et al.*³ showed that pseudohermaphrodite rats, thought to have a corresponding defect, similarly failed to respond to androgens.

Although the condition in both man and rat is obviously a genetic one, it has not yet proved possible to determine whether it is caused by an X-linked gene or by an autosomal dominant sex-limited to males in expression⁷. This report describes a condition in mice which closely resembles human testicular feminization, and which shows clear X-linked inheritance.

X-linked Inheritance of Testicular Feminization in Mice

A female mouse heterozygous for the X-linked genes tabby (*Ta*) and blotchy (*Blo*) and of genotype *Ta Blo/+*, when mated to a normal male, produced ten apparently female progeny which expressed the *Ta* and *Blo* phenotypes of hemizygotes or homozygotes. On dissection they proved to have testes, and chromosome counts showed them to have forty chromosomes. It was therefore thought that they must be genetic males with chromosomal constitution XY, and this was further confirmed by the sex ratio among the original female's offspring. Including the ten anomalous animals as females, there were fifty females and twenty-one males, a significant deficiency of

normal males. The similarity to testicular feminization in man was immediately obvious, and the gene postulated to be carried by the feminized males was given the name and symbol testicular feminization, *Tfm*.

Of the twenty-one normal males produced by the original female carrier, only two were *Ta Blo/Y* and nineteen were *+/Y*, whereas there were ten phenotypic females presumed to be *Ta Blo/Y* and carrying *Tfm*. This suggested that *Tfm* was X-linked, and further work was directed to confirming the X-linkage, locating the gene on the X-linkage map, and obtaining *Tfm/Y* animals without other mutant genes (such as *Ta* and *Blo*) so that the gene effect could be studied in an otherwise normal animal.

Linkage Studies

If the condition was indeed caused by an X-linked gene, as it appeared, then in the absence of crossing over, those daughters of the original female which were *Ta Blo/+* would be expected to carry *Tfm*, and those which were *+/+* would not. Fourteen such daughters were bred from, three which looked *Ta Blo/+* and eleven which looked *+/+*. All three *Ta Blo/+* produced some outwardly female offspring with a hemizygous *Ta Blo* phenotype and testes, and were therefore shown to carry *Tfm*. Data from them were used for linkage studies (Table 2). The eleven *+/+* daughters were mated either to *Ta* or to *Blo* males; nine produced only *Ta +* or *Blo +* daughters and normal males, one proved to be chromosomally XO, and the remaining one (mated to a *Ta* male) produced some apparently female offspring not showing the *Ta +* phenotype, and which had testes. This last female therefore carried an X-chromosome in which the *Tfm* gene had been separated by crossing-over from *Ta* and *Blo*. Data from this female and her descendants (Table 1)

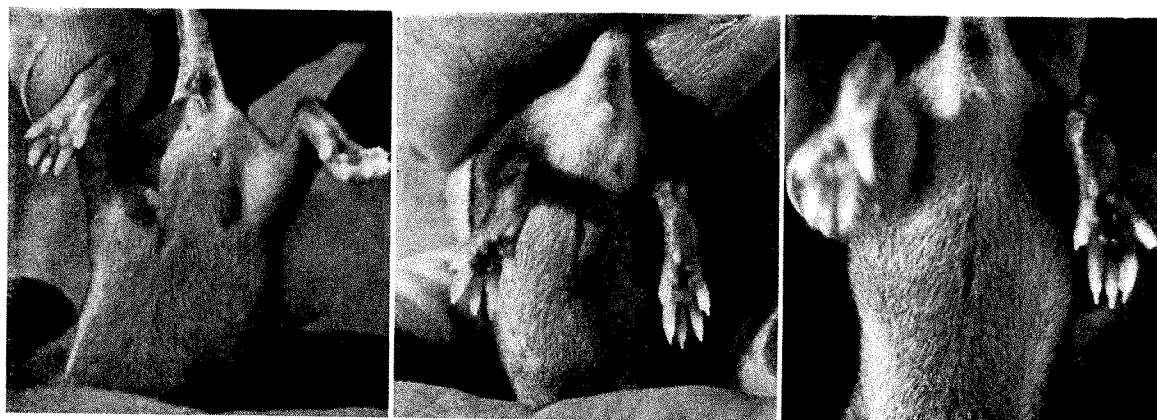


Fig. 1. Ventral view of 10-day old feminized male (*Tfm/Y*) mouse (centre) and normal female and male sibs, showing female appearance of *Tfm/Y*.

showed that *Tfm* (classified by lack of variegation in phenotypic females) segregated normally, except that there was some excess of genetically male (that is *Tfm* and phenotypic male) offspring throughout. The *Tfm* animals appeared to be of normal size and vigour.

Table 1. SINGLE-FACTOR SEGREGATION OF *Tfm*

Parent mating	Variegated females	Non-variegated females (<i>Tfm/Y</i>)	Males
<i>Tfm</i> +/+ × + <i>Ta/Y</i>	Obs. 32 Exp. 38.0	24 19.0	20 19.0
<i>Tfm</i> +/+ × <i>Ta</i> × <i>Blo/Y</i> or + <i>Gs/Y</i>	Obs. 64 Exp. 78.0	52 39.0	40 39.0

Table 2. LINKAGE DATA FROM MATINGS OF *Tfm Ta Blo* +/+ + FEMALES TO +*Gs* +/+ + MALES (*Tfm/Y* AND MALE YOUNG ONLY)

Phenotype of offspring	No.	Phenotype of offspring	No.
<i>Tfm Ta Blo</i>	35	<i>Tfm</i> + +	0
+ + +	38	+ <i>Ta Blo</i>	1
<i>Tfm Ta</i> +	1	<i>Tfm</i> + <i>Blo</i>	0
+ + <i>Blo</i>	3	+ <i>Ta</i> +	0
Recombination	No.	Per cent	
<i>Tfm</i> - <i>Ta</i>	1/78	1.3 ± 1.3	
<i>Ta</i> - <i>Blo</i>	4/78	5.1 ± 2.5	
<i>Tfm</i> - <i>Blo</i>	5/78	6.4 ± 2.8	

For the calculation of linkage (Table 2), only the *Tfm* and phenotypic male offspring were considered, because the true females could not be classified for *Tfm*. The least frequent pair of cross-over classes were *Tfm* + *Blo* and + *Ta* +, of which there were none. If these are considered to represent the double cross-over types, then the order of loci on the *X*-chromosome must be *Tfm* - *Ta* - *Blo*, with *Tfm* 1.3 ± 1.3 cross-over units from *Ta*.

Anatomy and Behaviour of Feminized Males

From birth until the normal time of sexual maturity, the feminized males were indistinguishable externally from normal females (Fig. 1). The ano-genital distance was normal for females, and the genital papilla was typically female in both size and shape. Normal teats were present, and the hair around the teats was short and yellowish, again as in normal females. In animals of this stock, the vagina usually opened at about 5 weeks, and from this age until about 3 months the *Tfm/Y* animals could be distinguished from normal females by their lack of a vaginal opening. In some but not all *Tfm/Y*, however, the vagina did open at about 3 months. Vaginal smears taken after this age yielded very few cells, mainly leucocytes, with a few nucleated epithelial and cornified cells.

Several *Tfm/Y* animals with open vaginas were left with known fertile normal males, but no vaginal plugs were observed. On the other hand, the feminized males were not observed to fight with normal males. Thus, it seems likely that their general behaviour was that of non-oestrous females. Normal males excrete a pheromone in the urine, the smell of which may be very obvious to the human nose in cages occupied by males. This smell was never noticed from cages of *Tfm/Y* mice.

Internally there were very small testes, lying posteriorly in the abdomen among the fat. The vas deferens and epididymis were absent and there were no male accessory glands. Neither were there female reproductive organs, except for the small vagina already mentioned.

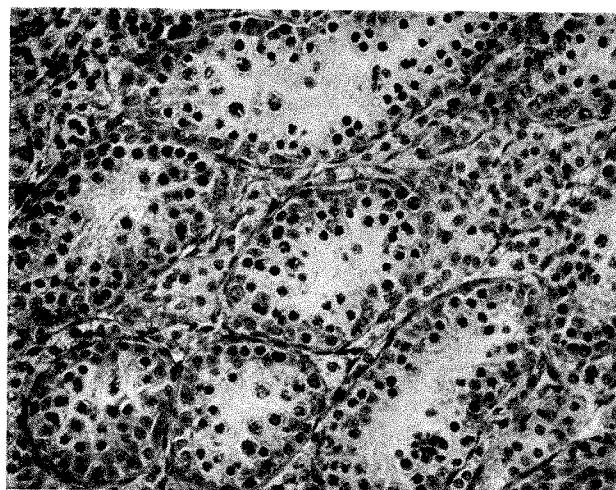


Fig. 2. Testis section of *Tfm/Y* mouse showing spermatogenesis arrested at spermatocyte stage, and hypertrophy of Leydig cells.

Testis Histology

Testes of animals aged from 3 weeks to 7 months were fixed in Heidenhain's Susa, sectioned and stained with haematoxylin and eosin. Spermatogonia, some in mitosis, and Sertoli cells were present in all (Fig. 2) but spermatogenesis ended at the spermatocyte stage or earlier. Some tubules contained meiotic prophase, and occasionally cells interpreted as meiotic metaphases were seen. As well as this, in some tubules, there were necrotic cells in the lumen. The tubules were typically hyalinized, and in the older animals the Leydig cells appeared to be hypertrophied, although no quantitative studies were made.

For chromosome studies, the testes were prepared by Meredith's method⁸, using the whole testis for one slide, because of its small size. Normal spermatogonial mitoses, with a modal chromosome number of forty, were found in all of eight animals, but in only two of these were first meiotic metaphases found, again with a normal chromosomal complement, of twenty bivalents, including a typical XY pair (Fig. 3).

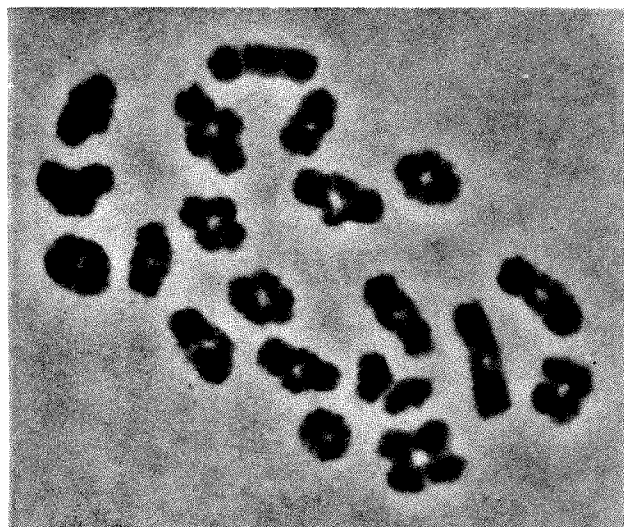


Fig. 3. Normal first meiotic metaphase from *Tfm/Y* mouse aged 6.5 weeks.

Similarities with the Condition in Man and other Mammals

The first question to be considered is whether this condition is indeed homologous with testicular feminization in man and other species. Anatomically the mice resemble the affected humans and rats in the absence of all male and female reproductive organs except the testes and vagina. In testis histology, however, there are differences, because in the mice dividing spermatogonia and early spermatocytes were always present, whereas, in humans, spermatogenesis is in most cases completely absent, although in some individuals spermatogenesis even up to the stage of mature sperm has been claimed¹. Leydig cell hyperplasia is found in all the species. Thus, anatomically and histologically, the similarities heavily outweigh the differences. The chief criterion, however, must be the biochemical one, because, in both humans and rats, the anatomical and histological defects are thought to be secondary to the biochemical defect of end-organ insensitivity to androgens^{2,3,5}.

It has been suggested that the insensitivity results from failure to convert testosterone to dihydrotestosterone, postulated to be its active metabolite⁹; however, this point is in doubt because neither affected humans nor rats showed any more response to administered dihydrotestosterone than to testosterone^{3,10}. The affected *Tfm* mice fail to respond to at least one of the actions of testosterone, that of inducing alcohol dehydrogenase (ADH) activity in the kidney^{11,12}. Moreover, it is unlikely that this lack of response is related to failure to form dihydrotestosterone, because this substance does not induce kidney ADH activity in normal mice¹⁸. It is also suggested that the *Tfm* gene is a non-inducible mutation of a repressive regulator which controls the structural genes for testosterone-inducible enzymes¹⁸. Thus it is likely that the mouse condition is indeed homologous with testicular feminization in rats and humans.

Is the Gene for Testicular Feminization in Man also X-linked?

The next question is whether one may safely deduce from this apparent homology, and from the fact that the *Tfm* gene in the mouse is X-linked, that the gene for testicular feminization in man is also X-linked. Ohno has postulated¹³ that the X-chromosomes of all mammals are homologous and that genes X-linked in one species are X-linked in all. Numerous examples supporting this hypothesis can be adduced, and none against. The number of genes known on X-chromosomes of various species is, however, not yet large enough to establish with certainty that a gene X-linked in one species must also be so in another. Rather one may say that the fact that *Tfm* is X-linked in the mouse greatly increases the probability of X-linkage of the corresponding genes for testicular feminization in man and in the rat and, moreover, that all mammalian X-chromosomes carry a gene with homologous function.

In the context of X-linkage it is interesting to consider the testis histology and the possible function of the *Tfm* gene in the testes. The functions of the X and Y chromosomes in mammalian spermatogenesis are at present unknown. In late spermatogonial and in meiotic stages both the X and the Y are condensed, late replicating¹⁴ and do not label with ³H-uridine¹⁵, suggesting that they are genetically inactive. In chromosomally XXY males of all mammalian species in which they have been so far found, spermatogenesis is, however, completely lacking, apparently because of the death of spermatogonia. This suggests that the X chromosome does have some function in spermatogenesis, and, moreover, raises the possibility that in early spermatogonia all X chromosomes present may be active, as is apparently the case in female germ cells¹⁶. If germ cells with two active X chromosomes die in the male but survive in the female, the question arises whether a response to testosterone, and therefore the *Tfm* gene, is involved. The histological studies have shown that spermatogonia of *Tfm/Y* mice survive and multiply. There is therefore no evidence that the normal functioning of the *Tfm* locus is required in spermatogonia. The germ cells of *Tfm/Y* then die at the spermatocyte stage. This is, however, not necessarily directly caused by the *Tfm* gene, because in genetically normal males cryptorchidism interrupts spermatogenesis at precisely this point¹⁷. The arrest of spermatogenesis in *Tfm/Y* may therefore be an unspecific secondary effect of cryptorchidism, rather than a result of defective gene action in the spermatogenic cells themselves. Thus there is no clear evidence at present that the action of the *Tfm* gene locus is required in mouse spermatogenesis, at least up to the spermatocyte stage.

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Growth of the Bacterial Cell

by

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Cell growth and division, and the way in which chromosome segregation is coordinated with these events, are discussed in terms of a model based on the concept of a unit cell—the smallest viable bacterium.

GENERAL interest in the way in which the bacterial cell membrane grows was stimulated by the suggestion that bacterial chromosomes are attached to the membrane, and that the growth of this membrane could therefore play a part analogous to that of the mitotic spindle in eukaryotic cells¹. Jacob, Brenner and Cuzin¹ put forward a model in which the assumption was made that the cell membrane grew only in a central zone, between the presumed points of attachment of two sister chromosomes. Cell division in the centre of this zone would result in the segregation of the two chromosomes into the two sister cells. Since that time, much evidence has accumulated to support the idea that the bacterial chromosome is indeed attached to the cell membrane²⁻⁴. It has proved much more difficult, however, to obtain information about the mode of growth of the bacterial cell membrane and little is known about it at present. We believe that the experiments here show the way in which the cell membrane must grow.

No technique has yet been devised for the direct examination of the sites of membrane growth in *Escherichia coli*, and we have therefore resorted to two indirect methods to study the growth of these cells. The first method is simply to measure the direction of growth of intact living cells relative to fixed external markers in the medium in which they lie⁵. The second is to measure the position of an internal marker relative to the ends of the cell. The internal marker which we have used is the point at which the cell wall splits in the presence of penicillin⁶. Both sets of observations indicate that growth is asymmetrical in cells which are less than a certain critical length. In longer cells, growth is symmetrical. To explain these results we suggest that a "unit cell", which is the smallest cell of a given strain that can exist in any growth conditions, has a single membrane growth site located at one pole. Formation of new membrane takes place on one side of this site, so that the unit cell grows at one end only. The number of such sites increases in proportion to the size of the cell, such that the number of sites is equal to the number of unit cell multiples in a cell of any given size.

If the proposed membrane growth sites were also the sites of attachment of the bacterial chromosomes, then the suggested mode of growth of the cell membrane would serve to ensure the accurate segregation of chromosomes at cell division.

Cells (*E. coli* 15T- JG151 or B/r) were grown in shaken cultures at 37° C in either minimal medium (M9 salts + glycerol ± thymine), enriched medium (M9 salts + glucose + casamino-acids + tryptophan + uracil ± thymine) or L broth (containing, in 1 l., 10 g tryptone, 5 g yeast extract and 10 g NaCl).

Apparent Direction of Cell Growth

Cells (from log-phase cultures) were placed on the surface of solid medium (growth medium + 1.5 per cent agar), a coverslip was placed over them, and their growth was observed with a microscope⁶. The positions of the ends of the growing cells were measured on an eyepiece scale (or on photographs) relative to the location of polystyrene latex spheres embedded in the agar.

Elongation of Cells in Minimal Medium

When the elongation of cells is followed on minimal medium it is clear that growth is almost invariably unidirectional. One end of the cell remains stationary relative to the agar surface, and the doubling in length of the cell takes place entirely by extension of the other end. Fig. 1 shows this pattern for a representative cell. (Cells completely embedded in soft agar behave in the same way, showing that unidirectional growth is not the result of cells adhering to the agar at a single point.)

Immediately after completion of the first cell division, the two sister cells slip out of alignment with one another (see Fig. 1), so that the two newly formed cell ends are no longer directly opposed. The two cells then grow in opposite directions to each other by unidirectional elongation at the newly formed ends. No further elongation takes place at the end of the cell which was moving in the parent cell. The two sister cells grow unidirectionally in opposite directions until each has doubled its initial length. The two sister cells which are now equal in length and parallel to one another then divide centrally. This pattern can be seen in the photographs in Fig. 1.

It seems therefore that, in minimal medium (with a 60 min generation time), cells grow by unidirectional elongation of one end only, and that this end is the one which was formed at the previous division.

Elongation of Cells in Rich Medium

A different pattern of growth is seen when cells are growing in rich medium. In L broth (generation time 24–30 min) single cells grow by elongation in both directions relative to the agar surface. After cell division, the two sister cells again move slightly out of alignment with one another, and continue to elongate by extension both of the newly formed end and of the old end.

Position of the Penicillin-sensitive Site

Schwarz, Asmus and Frank⁶ have shown that cells of *E. coli* growing in a rich medium are lysed in the presence of penicillin by the splitting of the cell wall at a single site. In these conditions, the point where the wall split was reported to be always in the centre of the cell. We have used a modification of the procedures of Lederberg⁷ and of Schwarz *et al.*⁶ to measure the position of this site of sensitivity to penicillin as a function of cell length. A range of cell sizes was obtained by growing cells in a number of different media (above). In this way it was possible to obtain a continuous range of cell lengths from approximately 1.7 to 6 μ m. To measure the site of penicillin sensitivity, log-phase cells were spread on agar layers containing growth medium (of the same composition as that in which the cells had been growing), 0.015 M MgSO₄ and penicillin⁷. The concentration of penicillin (417 IU/ml.) was sufficient to stop cell growth immediately. In these conditions, the cell wall splits and a stable spheroplast is extruded at the site of the split. The spheroplast is seen as a well-defined sphere attached to the rod-shaped cell wall at a single point (see Fig. 5). The position of this attachment point, relative to the two poles of the cell wall, was then measured on a photomicrograph.

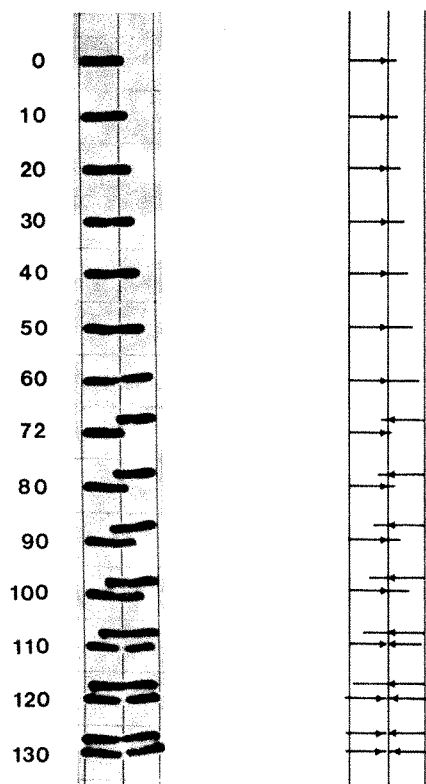


Fig. 1. The growth of a single cell of *E. coli* 15T⁻ JG151 on minimal agar (phase contrast pictures of living cells). The positions of the ends of the cells were measured at intervals, relative to fixed markers in the agar, as described in the text. The numbers on the left are the times (in min) at which the adjacent photographs were taken. The three vertical reference lines show the positions of the left end of the cell, of the division site and the right end of the cell at the time of division. The cell grows by elongation of one end from 0–60 min. It divides between 60 and 72 min. The daughter cells then slip out of alignment and each continues to grow unidirectionally from the end which was formed at the division. At 110 min one of these daughter cells divides and at 130 min the other, slightly slower growing cell, also divides. (At the second division the proximity of the sister cell prevents the two halves of the newly divided cell from slipping past one another. Subsequent growth therefore pushes the outer ends of the cells over the reference lines.) On the right of the figure, lines are drawn to represent the length and position of each cell. The arrowheads represent the position and direction of growth of the proposed growth sites (see text). The vertical reference lines are each 1.7 μ m apart.

It was found that the position of spheroplast emergence, relative to the ends of the cell, is a function of cell length. For cells of lengths between about 3.4 and 6 μ m this site is in the middle of the cell, but in cells shorter than 3.4 μ m, the position of this site is not central. Instead, the penicillin-sensitive site is always approximately 1.7 μ m from one pole of the cell. Thus in the shortest cells, themselves about 1.7 μ m long, the spheroplast emerges at one pole. As cell length increases, the location of the site is progressively further from the end until, at a length of 3.4 μ m, it becomes central. This relationship is shown in Fig. 2. The figure shows the mean length (\pm one standard deviation) of cells, plotted against the distance between the penicillin-sensitive site and the nearer end of the cell. (In cells which were visibly in the process of division, the spheroplast always appeared at the division furrow. Such dividing cells are not included in Fig. 2.)

Cells longer than about 7 μ m are not normally found in even the richest medium. To obtain longer cells, cell division was prevented by the addition of very low concentrations of penicillin. At a concentration of 15 IU/ml., both total cell mass (measured in terms of absorbance) and total DNA continued to increase exponentially at the same rate as in control cultures, but cell division was completely suppressed. In this way cells were obtained with lengths up to 65 μ m.

When such long cells were placed on agar containing a high concentration of penicillin (417 IU/ml. + MgSO_4), spheroplasts formed as readily as in shorter cells. Most of these long cells produced only a single spheroplast, but this could be located either in the centre or at about 25 per cent from an end. Clearly this might mean either that a given cell has two possible penicillin-sensitive sites (one in the middle and one at 25 per cent from one particular pole) or three such sites (at 25, 50 and 75 per cent of the cell length). The method used by us cannot distinguish between these two possibilities, but we believe that there are in fact three sites per cell, for reasons that are given below. The location of the penicillin-sensitive sites in cells of various lengths is shown in Fig. 3. When the data are plotted in this way the measurements cluster mainly around two lines.

Penicillin-sensitive Sites and Division Sites

There is much evidence to suggest that penicillin-sensitive sites are located either at division sites or at potential division sites. First, dividing cells are more sensitive to killing by penicillin than non-dividing cells^{7,8}. Moreover, in dividing cells the site of splitting of the cell wall is always at the division furrow itself. Second, inhibition of DNA synthesis, which is known to inhibit cell division⁹, also prevents the splitting of the cell wall in the presence of penicillin⁶.

Further evidence that penicillin-sensitive sites and potential division sites occupy identical positions in the cell is provided by measurements of the location of division sites in long cells. The number and position of division sites in long cells (produced by growth in low concentrations of penicillin) were obtained by adding penicillinase to destroy the penicillin, after which cell division recommences¹⁰. After addition of penicillinase, the cells were placed on agar and photographed as divisions occurred. The distribution of these division sites (Fig. 4) is the same as that of the penicillin-sensitive sites in cells of the same lengths (Fig. 3).

Generally only a single spheroplast is produced by a cell of any length. We assume that this results from reduction of pressure within the cell wall as soon as the first spheroplast emerges. A single division does not,

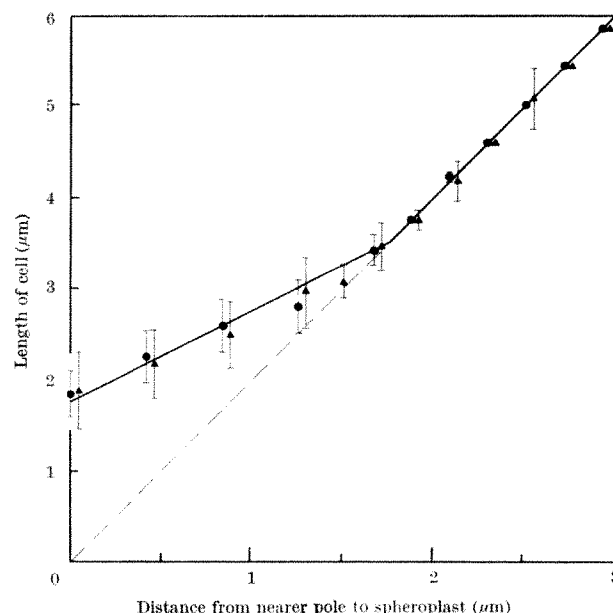


Fig. 2. Means and standard deviations of cell length (ordinate) plotted against the distance from the point of spheroplast emergence to the nearer of the two ends of the cell (abscissa). Lengths and distances are in μ m. \bullet , *E. coli* B/r; \blacktriangle , *E. coli* 15T⁻ JG151. For clarity these points are displaced a short distance to the right of the points for B/r. Measurements of 513 single cells of B/r and 880 of 15T⁻ JG151.

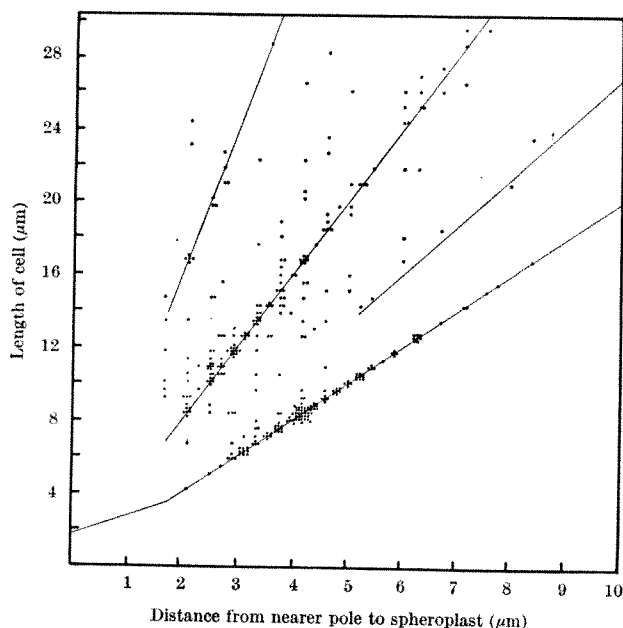


Fig. 3. Relationship between position of the penicillin sensitive site and cell length in long cells. Scales as in Fig. 2. Data from cells of *E. coli* B/r in which division had been inhibited by low concentrations of penicillin (see text). Individual measurements are shown, rather than means, because the points cluster about at least two lines. The scatter in the measurements arises from the difficulty of determining the precise site of emergence of a spheroplast. The lines show the theoretical location of potential division sites, calculated from the model shown in Fig. 7.

however, preclude subsequent divisions at other potential division sites in the same cell. Consequently the positions of cell division sites in a cell can be located by continuing to observe a long cell until it has divided up at all potential division sites. In this way we were able to show that there are three potential division sites in cells over about 7 μm in length (and seven in cells over 14 μm). It is for this reason that we believe that the number of penicillin-sensitive sites in cells of this length is also three.

Penicillin-sensitive Site and Site of the Preceding Cell Division

The two poles of a bacterial cell are not identical in their past histories. One of the two poles was formed during the most recent cell division, while the other is older. We have been able to determine the spatial relationship between the position of the penicillin-sensitive site and the "older" and the "younger" poles of the cell. This relationship is important for the construction of our model of cell growth.

The younger of the two cell poles may be identified by following the growth and division of individual cells on agar. A cell is observed until division has taken place. The two sister cells are then allowed to grow for a variable period of time (always less than a generation time). The younger poles in such cell pairs are therefore always known. To determine the position of the penicillin-sensitive site relative to the older and younger poles, a high concentration of penicillin (10,000 IU/ml.) is introduced into the vicinity of this pair of sister cells by way of a pre-cut channel in the agar. In this way the position of the cells on the agar is not altered, while the rapid diffusion of the penicillin into the agar stops further cell growth immediately. Spheroplasts then form on the cells in the usual way and the position of the penicillin-sensitive site can be measured relative to the known younger and older pole of each cell.

Fig. 5 shows the formation of spheroplasts in such a pair of sister cells. In each sister cell, the penicillin-sensitive site was closer to the younger pole of the cell and 1.7 μm from the older pole. Measurements of this

kind have been made on forty-five individual cells. The penicillin-sensitive site is always closer to the younger end of the cell and, on average, 1.9 μm from the older pole.

A Unit Cell Model of Bacterial Growth

We may summarize our observations on the growth of cells of *E. coli* as follows. (1) The growth of cells is always unidirectional if they are less than a certain critical length (about 3.4 μm) and always bidirectional if they are more than this length. (2) When growth is unidirectional, extension always takes place from the pole of the cell that was formed in the preceding cell division. (3) The penicillin-sensitive site, visualized as the point at which a spheroplast is formed, is never less than about 1.7 μm from the older of the two poles of the cell. (4) The number of penicillin-sensitive sites, together with the number of potential division sites, increases from one per cell to three per cell when total cell length exceeds about 6.8 μm . The locations of the two kinds of site are identical and these locations are spaced at equal intervals along the length of the cell.

To provide a conceptual framework for this set of observations, we have developed a simple model of cell growth. According to this model, cells of *E. coli* have a minimum length of about 1.7 μm . We shall refer to a cell of this length as a "unit cell". A unit cell has a single membrane growth site located at the pole of the cell which was formed at the previous cell division. Net synthesis of new membrane takes place asymmetrically at the growth site, so that during growth this site remains at a fixed distance (1.7 μm) from the older of the two poles of the cell. Consequently, when the cell has grown to twice its initial length (that is, to two unit cell lengths) the growth site is in the centre of the cell.

If the cell divides at this length (as it will do if the cell is in minimal medium) the division furrow will pass through the position of the growth site. Each of the two daughter cells is then assumed to receive a growth site at this newly formed pole and therefore to commence growth at this pole. Because such sister cells are now observed to grow in opposite directions from one another, it is assumed that the two newly formed growth sites have opposite polarities to each other.

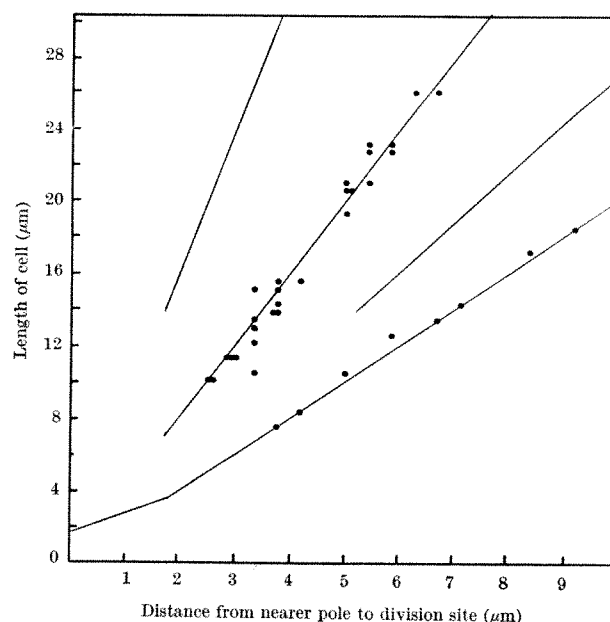


Fig. 4. Location of the sites of first cell division, after the addition of penicillinase to long cells in which division had been inhibited by low concentrations of penicillin. Scales as in Fig. 3. (*E. coli* 15T- JG151.)

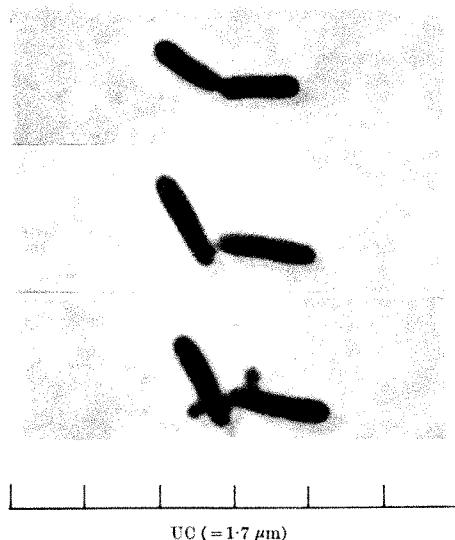


Fig. 5. Position of the penicillin-sensitive site in relation to the newly formed pole in sister cells. Cell division is shown in the top photograph. When the sister cells had separated and grown for a short time, a high concentration of penicillin was added to stop growth and induce spheroplast formation (middle). The emergence of the spheroplasts is seen in the lower photograph. (*E. coli* 15T- JG151 on minimal agar.)

The workings of this model are illustrated on the right-hand side of Fig. 1, with reference to the successive stages in the growth of the cells shown to the left of the figure.

In the example considered here, cell division took place at two unit cell lengths. But if the original unit cell had been placed in a different growth medium, such that it did not divide on reaching this length (for example, if the medium contained a low concentration of penicillin), then we assume that the duplication of the growth sites takes place exactly as before. When the cell reaches 2 unit cell lengths, therefore, two growth sites of opposite polarities would form in the centre of the cell. In this case the two sites would not be in two separate cells, so that the result of growth taking place at each of them would be that all new material would be laid down as a central zone. The cell as a whole would therefore extend in length equally in both directions. In cells growing in conditions in which the length of a newly divided cell is always equal to or greater than 2 unit cell lengths, such as is the case for cells growing in L broth, growth will be bidirectional at all stages of the cell cycle. Fig. 6 illustrates this model of cell growth.

Sites of cell division are assumed to arise at the time of the duplication of the growth sites. Thus, whenever a pair of new growth sites is formed, a site of potential cell division is assumed to be formed from the old growth site. Such a mechanism will result in the number and location of division sites which we have observed in cells of different lengths. Fig. 7 shows the predicted locations and numbers of growth sites and potential division sites in cells of lengths from 1 to 16 unit cell lengths. The observations on number and location of penicillin-sensitive sites and division sites are in good agreement with this model of cell growth.

This model of cell growth can be seen as a logical extension of the model first proposed by Jacob, Brenner and Cuzin¹. That model was suggested in the first place to provide a mechanism by which the spatial segregation of copies of the bacterial chromosome could be coordinated with cell growth. Our unit cell model of growth can also provide such a mechanism. Thus, coordination between cell growth and the timing of chromosome replication will be achieved if the initiation of rounds of DNA synthesis takes place whenever the number of unit cells doubles. At this time a single round of chromosome

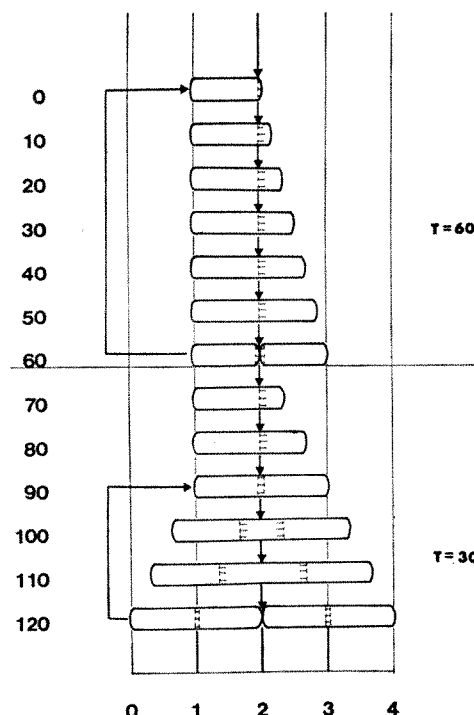


Fig. 6. Model of cell growth. In the imaginary experiment depicted, a unit cell is grown for one cell cycle in a medium where the mass doubling time is 60 min ($T=60$). At 60 min the daughter cell on the left is transferred to a richer medium where the mass doubling time is 30 min ($T=30$). The normal cell cycle for cells growing continuously in these two media is shown by the groups of cells connected by the heavy arrow to the left of them. During a shift up of the kind shown here, however, there will be a 60 min interval between transfer to the new medium and the next cell division (see ref. 11). In consequence, the length of cells at this division will be twice the length of dividing cells in the old medium. (For simplicity the increase in cell volume after the shift up is assumed to result from increase in length without increase in diameter.) The growth sites are shown as dashed vertical lines across the cells, and the direction of growth at each site is shown by the horizontal lines attached to them. Each growth site gives rise to two new sites of opposite polarities when the cell reaches a length of 2 unit cells. The central vertical line (line "2") corresponds to the spatial location of cell divisions. The triangles show the penicillin-sensitive sites.

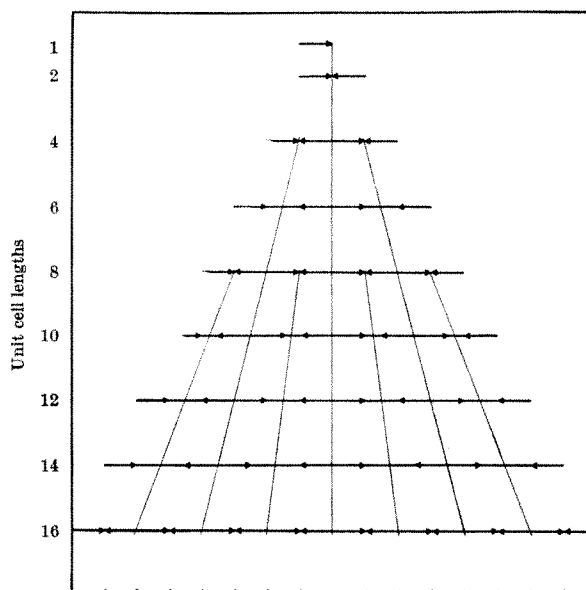


Fig. 7. Model of cell growth for long cells. The diagram shows the number and location of growth sites (arrowheads) in cells of different length (horizontal lines). Lengths are given in unit cell lengths (1 unit cell length $\approx 1.7 \mu\text{m}$). The direction of the arrowhead gives the direction of membrane growth at that site. Each growth site gives rise to two new sites of opposite polarities, together with a potential division site, whenever the number of unit cell lengths doubles. The locations of the potential division sites are therefore given by the points of intersection between the horizontal lines and the thin lines.

replication is initiated in each unit cell. The mass of a unit cell must therefore equal the "initiation mass" (M_1) which must be reached before new rounds of DNA synthesis can begin¹². In a cell of any given size, the ratio between the number of unit cells (or number of growth sites) and the number of chromosome origins will therefore always be one. To obtain spatial partition of chromosomes into sister cells, it is necessary to assume that the chromosomes are physically attached to the cell membrane at the growth sites. We assume that chromosomes are attached to these sites at their replication forks⁴. Initiation of new rounds of DNA synthesis therefore results simply from the appearance of new attachment sites where such replication can take place. In the case where new rounds of chromosome replication are initiated before the previous rounds are complete (dichotomous replication) we assume that DNA synthesis continues at the "old" growth site as well as being reinitiated at each pair of "new" sites. This physical association between the replicating chromosome and what is now a potential division site is seen as the reason why termination of each round of chromosome replication is a necessary prerequisite for cell division^{13,14}.

This unified model of cell growth is, so far as we can see, consistent with present knowledge of the bacterial cell cycle. In particular, the one to one correspondence between the number of growth sites (old + new), calculated according to our unit cell model, and the number

of chromosomal replication forks, calculated according to the model of Cooper and Helmstetter¹¹, is strongly suggested by present evidence. Other predictions of the model, such as the association between these replication forks and discrete membrane growth sites, remain to be demonstrated.

Measurements of the position of the penicillin-sensitive site in *Pseudomonas aeruginosa* have shown that the pattern of cell growth in this unrelated bacterium is identical to that reported here for *E. coli* (unpublished observations of W. D. D. and K. J. B.). It is probable therefore that this mode of growth is common to at least the Gram-negative bacteria.

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How the Cerebellum may be Used

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Recent anatomical information suggests new input-output relations for the cerebellum. These have interesting implications about the role of motor cortex in the learning and controlling of voluntary movements.

THE vertebrate cerebellar cortex has a very uniform structure, and may, for the purpose of this article, be regarded as being composed of many units like that appearing in Fig. 1. Its only output is the projection of large inhibitory cells, the Purkinje cells (*Pu*), to the intracerebellar nuclei, and to some of the vestibular nuclei^{1,2}. In man, a major projection from the intracerebellar nuclei is to the ventro-lateral nucleus of the thalamus (*VL*)^{1,2}. *VL* cells project to the motor cortex.

There are two kinds of input to the cerebellar cortex: the mossy fibres, which synapse with the numerous granule cells; and the climbing fibres, which project directly to the Purkinje cells and wrap themselves around their dendrites. Each Purkinje cell receives one climbing fibre¹, and can be powerfully excited by it. The climbing fibres arise from a group of cells in the contralateral brain stem¹; the curious shape of this group has led to its being named the olive. The inferior olive (*IO*) receives connexions from a wide variety of sources, in particular from the cerebral cortex³. The mossy fibres have several different sites of origin²; particularly important are the pontine nuclei (*PN*) of the brain stem. The cerebellar granule cells, with which the mossy fibres synapse, send axons (the parallel fibres) to the Purkinje cells, and to the inhibitory interneurons of the cortex.

In a recent article³, it was shown that the known anatomy and physiology of the cerebellar cortex are

consistent with its interpretation as a simple memorizing device. It was predicted that the synapses between parallel fibres and Purkinje cells are modifiable, being facilitated by the conjunction of pre-synaptic and climbing fibre activity. It was shown how this would allow any single Purkinje cell to learn to recognize, without appreciable confusion, more than 200 different mossy fibre input patterns. Two methods were outlined by which such a memorizing device might learn to perform motor actions and maintain voluntary postures initially organized elsewhere. Since then, three relevant facts have come to our attention: (i), anatomical information concerning the origin of the cortico-olivary and cortico-pontine projections⁴; (ii), the discovery that the olivo-cerebellar (that is, climbing) fibres branch^{5,6}; and (iii), the prediction that climbing fibres can organize more than simple memorizing phenomena⁷. These facts have implications about the way the cerebellum may be used by the rest of the nervous system that will be of interest to experimenters, and we therefore give here an outline of their principal consequences.

New Information

(i) The origin of the descending projection to the olive has long been known to include cortical cells, of which the majority lie in the motor and pre-motor areas. But it has recently been shown that these fibres arise almost

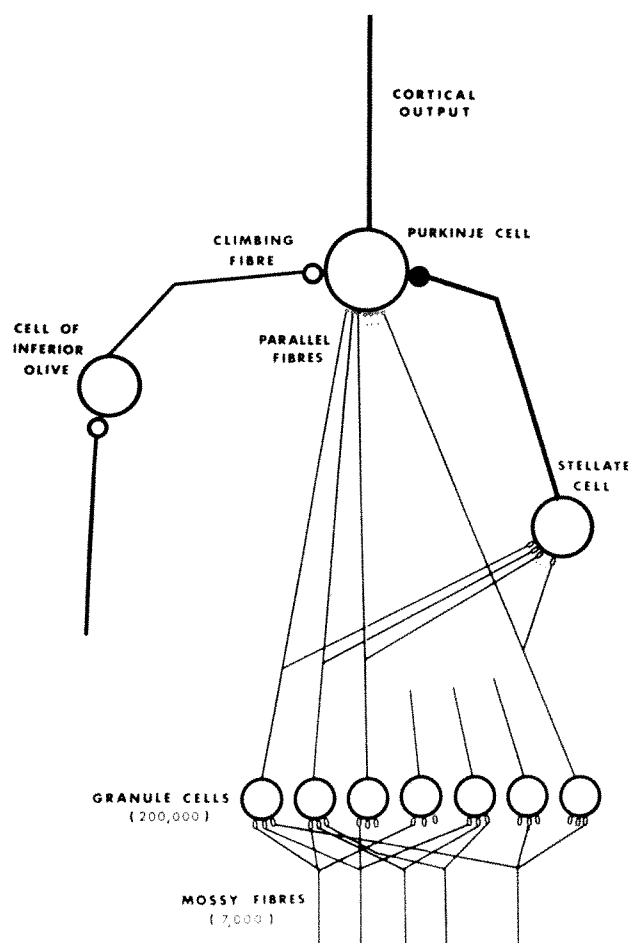


Fig. 1. The diagram selects the principal elements of the cerebellar cortex. There is one output system, the Purkinje cell axons, and two input systems, the climbing and the mossy fibres. The climbing fibres originate in the inferior olivary nucleus, and each Purkinje cell usually receives exactly one. The mossy fibres, which come from many parts of the body and brain, are imagined to convey information about the state of the animal—information referred to as the “context” at that time. The mossy fibre input is translated by the granule cells into a language of subsets, and the granule cell axons become the parallel fibres. It is predicted that the parallel fibre synapses with a Purkinje cell that are coactive with its climbing fibre are facilitated. The inhibitory cell prevents the Purkinje cell from firing unless almost all its active afferent synapses have been facilitated. The numbers of the various kinds of fibre projecting to one Purkinje cell in cat are as shown: this enables a single cell to learn at least 200 different mossy fibre inputs, without confusion between learned and unlearned events.

entirely from small pyramidal cells⁴. In contrast, the pontine nuclei receive collaterals from both large and small pyramidal cells⁴. The distinction may be that superficial pyramidal cells project to the inferior olive, while deep pyramidal cells give off collaterals to the pontine nuclei on their way to the spinal cord^{4,8}. Further, the projection from the ventro-lateral thalamic nucleus to the motor cortex is direct to the deep pyramidal cells, and perhaps by way of an excitatory interneurone to both the superficial and the deep pyramids (see Fig. 1^{2,8,9}).

(ii) The inferior olive contains fewer cells than there are cerebellar Purkinje cells¹⁰. This means that either there are other sources of climbing fibres or the olivo-cerebellar fibres branch. It seems that the latter explanation is correct^{5,6}. The distribution of the branches of one climbing fibre also seems to be restricted to a parasagittal plane⁶.

(iii) The hypothesis³ that the parallel fibre–Purkinje cell synapses are facilitated by simultaneous pre-synaptic and climbing fibre activity has implications deeper than merely allowing each Purkinje cell to memorize 200 or so different mossy fibre inputs. If a number of similar mossy fibre inputs have been learned and later an unlearned

input is presented which is near enough to those which have been learned, then the Purkinje cell may treat the new input as if it had been learned. This is probably not the disadvantage it was once thought³. It means that a Purkinje cell will generalize its response to all events in those regions where learned events are sufficiently clustered together. The implications of this generalization are set out elsewhere⁷.

Consequences of this New Information

Input-output relations. In Fig. 2, the new information (i) is combined with the previous knowledge of cerebellar anatomy. All the synapses in the diagram are excitatory, except those from the Purkinje cells to the cells of the cerebellar nuclei. One very striking feature of this circuit diagram is the loop formed from the deep pyramids through the pontine nuclei, cerebellar nuclei and VL nucleus of thalamus back to the deep (and also superficial) pyramids. This arrangement has been commented on before^{1,11}. A necessary assumption of the present theory is that this loop, which will provide a positive feedback from the deep pyramids to themselves, is so arranged as to give rise to temporally extended pyramidal cell outputs. One possibility would be that the feedback is chiefly to the original area, so that a movement—once initiated—will tend to continue indefinitely (at least well beyond the normal firing period of pyramidal cells in response to an excitatory input); and this will only be terminated either by applying direct inhibition to the deep pyramidal cells or by breaking the feedback loop. In the original cerebellar theory³, two possible forms of input-output relation were described, both of which required that each individual Purkinje cell could initiate one of the elemental movements into which it was postulated all actions were broken down. For executing actions it was thought necessary only to copy the correct pattern of elemental movements. It was shown how the cerebellar cortex could arrange this by having every elemental movement driven by the context in which it is required.

The anatomy of Fig. 2 is not wholly compatible with this simple programme for copying patterned sequences of elemental movements. In general, if a machine has to execute a sequence of movements, it can operate either by turning on the correct elemental movements at any instant, or by turning off all the incorrect ones. We believe that the design of the cerebellum suggests that the second scheme, the converse of the original input-output relations, is in fact used for learning motor actions. The second scheme is at first sight absurd, because the number of elemental movements required at any instant is far smaller than the number of possible elemental movements. It only becomes more economical than the first scheme if the number required exceeds the number which need to be turned off. In practice, this means that some agency must, at any instant, select from the vocabulary of elemental movements a particular set of “possibles”, which includes all those actually required. If this can be done so that the number of “actuals” is greater than the number of “possibles” minus “actuals”, it becomes cheaper to operate by deleting unwanted elemental movements from the set of “possibles”.

Such an agency would have to satisfy the following properties: (a), it must consist of cells capable of driving elemental movements; (b), these cells must be capable of being context-driven; (c), the set of situations to which each cell responds must include those in which it is needed; and (d), cerebellar action upon it must be such that Purkinje cell activity turns off instructions for one (or more) elemental movements. We propose that the set of deep pyramidal cells in the motor cortex is such an agency, and that the conditions (a) to (d) are satisfied by them.

One can now assign a definite role to the small superficial¹⁸ pyramidal cells which project to the inferior olive.

plausible embryological model which ensures that the Purkinje cells related to one olivary cell all converge on a single cerebellar nuclear cell—so that there is a one to one correspondence between olivary cells and cerebellar nuclear cells. But such a restriction is by no means necessary for the theory.

Detection of clusters by Purkinje cells and climbing fibres.

It seems likely that two parts of the theory developed by one of us⁷ for the cerebral pyramidal cells also apply to the cerebellar Purkinje cells. The first concerns the nature of the signals which the Purkinje cells actually transmit. It is possible that these cells do give a response which is strictly all-or-none, depending on whether the current input has been learned. We feel, however, that it is more likely that they signal a measure of how similar their current output is to the structure of the events that they have learned. It seems that the most suitable measure of this similarity is the fraction of the currently active afferent synapses to a cell which have been modified⁷, provided that fraction is greater than some fixed lower bound p (say). A model has been proposed by which this quantity could be measured by a single cell⁷, and we feel that this is likely to be more suitable for the theory of Purkinje cell dendrites than the simple one developed earlier³.

This raises important questions concerning the need for convergence of Purkinje cell discharge onto cerebellar nuclear cells. Is it possible for a single maximally firing Purkinje cell to turn off a cerebellar nuclear cell completely, or does it need convergence from several Purkinje cells? And if several converging Purkinje cells are firing sub-maximally—in response to inputs rather dissimilar to their learned partial contexts—then is their summed effect sufficient to turn off the cerebellar nuclear cell?

The second application of the cerebral theory to the cerebellum concerns the discovery that a climbing fibre can organize a kind of cluster analysis⁷. Provided the information arriving at Purkinje cells is clustered and that the climbing fibre is coactive with enough events in a cluster, then the cell will respond to many more events, whether or not they have ever been associated with the climbing fibre activity. We think that this effect, certainly vital in the cerebral cortex⁷, is probably important in the cerebellum also. It is a mechanism which can provide a kind of generalization to events which should "obviously" initiate the same responses as their neighbours without the necessity for a specific new learning trial.

The next topic we wish to raise concerns the Purkinje axon collaterals¹. It has been pointed out³ that the effect produced by them through their connexions with basket and stellate cells is simple, whereas their effect through the Golgi cells is not. One possible explanation of their existence is that, when active in the region of a particular Purkinje cell P , they cause P to relax the scale on which it measures the similarity of the current input to the events it has learned. This is suggested by two facts: first, the inhibition reaching P will be decreased by collateral stimulation; and second, the Purkinje axon collateral inhibition of the Golgi cells will cause a slight decrease in the local granule cell threshold. This is the correct step for interpreting the current mossy fibre input within the structure formed by the other mossy fibre inputs which it has learned (by the interpretation theorem⁷).

It is therefore possible not only that direct generalization, of the sort described above, can occur in the cerebellar cortex, but also that the extent to which this generalization is permitted (that is, lowering the value of p) can be varied by Purkinje axon collateral activity. If this is so, it has implications about the distribution of these collaterals that one would expect to find: because the cues to lower p for a particular cell P must arise from information suggesting that it would be appropriate to do so. This means that the Purkinje axon collaterals ending in one region of cortex should fire only when it is likely that the corrections controlled from these are wanted;

and in general, the more likely they are to be wanted, the greater will be the permissible degree of generalization there (that is, the lower p can be), and so the more activity there should be in the Purkinje axon collaterals terminating there. This implies that the collaterals from each Purkinje cell P_1 tend to be distributed to regions of cortex containing Purkinje cells which are needed after or at the same time as P_1 . The most obvious of such regions would be those containing the Purkinje cells which are fired by the other branches of the olivo-cerebellar axon which sends a branch to P_1 . (It is interesting to note that Purkinje axon collaterals are often closely related to climbing fibres.) Those regions of cortex receiving collaterals from many currently active Purkinje cells would then be more likely to be needed next than those regions receiving from only a few. The known distribution of Purkinje axon collaterals tends to support this notion. The Purkinje axons first contribute collaterals to the transversely running infraganglionic plexus, whose fibres often bridge across several folia; branches are given off from this plexus to the longitudinally running supraganglionic plexus, whose distribution is much more limited. Hence Purkinje axon collateral effects will tend to be restricted to the parasagittal plane. We have already shown that there is reason to suppose that the Purkinje cells have closer relations to other Purkinje cells within such a plane than without.

There is one other piece of evidence in favour of this rather complex view of the Purkinje axon collaterals. It is that it also accounts for the climbing fibre collateral effects^{1,3}. For, during learning, any instruction to generalize must be annulled, in order that a true record of the mossy fibre input may be stored. According to the theory³, learning occurs at P when the relevant climbing fibre is also active; and when it is, the effect of its collaterals could roughly balance the effect of the Purkinje axon collateral near P . According to the available evidence¹, both types of collateral are weak and their effects are opposite.

Timing Relationships

We have argued that the small, superficial pyramidal cells of the cerebral cortex detect incompatibilities in the current deep pyramidal cell activity, and that they modify the behaviour of the cerebro-cerebellar-cerebral loop to cope with this. We now consider the timing relationships involved.

The speed of the main "feedback" loop is astounding. It incorporates some of the fastest pathways in the nervous system, and its major links all include monosynaptic connexions^{1,11}. In the cat, discharges in the pontine nuclei follow stimulation of the cerebral white matter by as little as 2 ms⁴. The corresponding times for the other stages are: pontine nuclei to cerebellar nuclei, 1 ms¹¹; cerebellar nuclei to VL nucleus of thalamus, 2 ms¹; VL nucleus of thalamus to cerebral pyramidal cells, an estimated 1 ms. The whole loop may therefore be traversed in as little as 6 ms, and certainly within 10 ms. Such a fast mechanism is clearly required in voluntary movements, especially those of a more complex kind when muscular groups have to be set into action in rapid sequence and at closely defined times.

Contextual information reaching the cerebellar cortex through the mossy fibres is also rapidly transmitted; indeed, it involves almost the same pathways. The time taken for stimulation of the subcortical white matter to evoke a mossy fibre response is 2.7 ms⁴. Mossy fibre responses to stimulation of forelimb and hindlimb peripheral nerves have delays as short as 5 ms and 7 ms respectively⁴.

On the other hand, the cortico-olivary-climbing fibre pathway is quite slow. The climbing fibre discharge evoked by stimulation of the cerebral subcortical white matter has a delay of 15 ms⁴. At first sight, it would therefore seem impossible that the superficial pyramidal

could signal that the currently active deep pyramids should be deleted: their commands would arrive too late to be effective.

It is, however, necessary to consider the time scale of the context in which these instructions are being made. The overall context of the movement changes much more slowly than the individual components of that movement. That a given group of deep pyramidal cells should not fire is not merely a decision whose effects last for a few milliseconds: the group will be required to be off for an extended period of time. The decision may have to be made and implemented quickly, but it will remain in force for much longer. This means that the modification conditions refer to extended contexts, of perhaps as long as 100 ms, rather than to instantaneous contexts.

It is therefore proposed that the inferior olive cells should fire in prolonged bursts, of up to 100 ms. During this time, the currently active synapses to the related Purkinje cells should be strengthened in proportion to their degree of activity. This allows the Golgi cell threshold system to be reset by the climbing fibre collaterals, so as to give the "correct" parallel fibre pattern during modification. More important, this ensures that the Purkinje cells can respond in good time to inhibit the cerebellar nuclei cells—because the mossy fibre context just before the climbing fibre activity (that is, when the input reaches the pontine nuclei) will differ only slightly from that during it. The ability of Purkinje cells to generalize will also help in this effect.

It may be found that the small, superficial pyramidal cells anticipate the large, deep pyramidal cells, and signal in advance that certain cerebellar nuclei cells must be inhibited within the context of the present developing movement.

Cerebellar Disorders

The present theory can provide a tentative explanation for many of the disorders arising from damage to the cerebellum. One of the most striking effects of acute cerebellar lesions is the delay in the initiation and termination of movements¹³. The delay in initiation is probably caused by malfunction of the cerebellar nuclei. In the acute stage of such lesions, there is considerable oedema and consequently raised pressure in the cerebellum; this could account for such malfunction. The result is that, when the cerebral cortex tries to initiate the movement, there is little or no excitatory feedback to the motor cortex. The movement can only be got going by a considerably greater voluntary effort, and this involves both delay and slow pick-up. With recovery of functioning of the cerebellar nuclei (that is, in those lesions which are more superficial), such delays will tend to disappear¹³.

Delay in termination¹³ probably results from a combination of two factors. First, there is an inability to initiate the muscular contractions which are required to stop the movement: this again involves the cerebellar nuclei. Second, there is delay in switching off the current movement: this results from the malfunction of the cerebellar cortex. This latter effect should become more apparent as recovery proceeds, for the cerebellar nuclei will be functioning normally while the damage to the cerebellar cortex persists. In other words, the context which signifies that the movement should stop is no longer able to implement this operation, because the relevant Purkinje cells are lacking. This argument receives support from the observations of Gordon Holmes¹³ that the start of relaxation in a movement is usually more markedly affected than the start of contraction.

The inability of patients with unilateral cerebellar lesions to maintain voluntary postures on the affected side, and the greater sense of effort involved in making any voluntary movements, are both common features in the early acute stages. Both are consequences of inadequate excitatory feedback from the cerebellar nuclei.

The phenomena of dysmetria¹³, in cases of acute

cerebellar lesions, and of hypermetria, which occurs in more persistent cases, are probably related to these disorders. Dysmetria will result from the malfunctioning of both cerebellar nuclei and cerebellar cortex. Movements, once initiated, are ill-gauged and tend to undershoot or overshoot the mark. Undershoot will be caused by an inability to maintain a voluntary movement (a symptom of cerebellar nuclei malfunction); overshoot will be caused by inability to stop voluntary movements (already considered). It is particularly interesting that hypermetria should ensue—this is exactly what the theory would predict. It results from the lack of inhibitory control from the cerebellar cortex; as a result the movements consistently overshoot and are excessively forceful.

The decomposition of complex movements¹³ is a natural consequence of any cerebellar malfunction. The errors arising in the initiation, continuation and termination of successive and concurrently running elemental movements should lead to hopeless confusion. The only hope for success would be to deal with one elemental movement at a time, so that errors may be consciously and deliberately dealt with as they arise.

An interesting disability which arises in cerebellar patients is that on trying to flex just one finger (in order to bring it into apposition with the thumb), they frequently flex all four fingers at the same time¹³. In this case, it may be that normally the cerebral command is to flex all four fingers but suppress flexion on the unwanted three. Certainly in early hand movements, flexion of all four fingers appears before flexion of individual fingers—though there is a cortical representation for each individual finger flexion. The suppression of the unwanted flexions is learned by the cerebellum during the early development of the child. Damage to the cerebellar cortex will interfere with the suppression, and a command to move one finger will initiate movement in all four.

We shall make just one reference to observations made on animals with lesions placed in the cerebellum. This concerns the effects of such lesions on the placing reaction¹⁴. Lesions which involve the dentate nucleus are found to abolish the placing reaction. In contrast, lesions confined to the cerebellar cortex may actually enhance it. Ablation of parts of the cerebral cortex which include the motor area is known to abolish the placing reaction. This is compatible with a learned reflex which passes through the cerebral motor cortex and whose output depends on positive feedback through the cerebro-cerebellar-cerebral loop. Clearly such a reflex is of use to the animal in standing and walking. Inhibitory control of this reflex is then exerted by the cerebellar cortex.

The functions of the ascending spino-cerebellar and spino-olivo-cerebellar tracts, and their utilization in the control of movements and postures, will be dealt with elsewhere.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Irregularities in the Crab Pulsar Wobble

THIS article presents new measurements of the arrival times of optical pulses from the Crab pulsar, NP 0532. The observations were obtained at Hamburg Observatory (Bergedorf) with a 60 cm refractor and a data acquisition system with an on-line computer (PDP-8/S). Comparison of our results with those obtained by Boynton *et al.*¹ and Richards *et al.*² does not support the assumption of a periodic wobble with a period of 77 days (ref. 2).

The arrival times for twenty-eight nights between October 10, 1969, and April 9, 1970, had a computed mean error for the mean arrival time for one night of 100 μ s or less. They were obtained with a pulse counting photometer (EMI 9781 tube) with a circular diaphragm with a diameter of 10 inches and no colour filter. The time of arrival for a pulse is derived from the light curve obtained from about two minutes of integration and is defined as the median³ of the photon distribution in an interval of ± 2 ms around the peak. This median is systematically 240 μ s earlier than the peak tip of the average light pulse⁴ due to the asymmetry of the pulse. A total integration time of 20 min or more was achieved each night.

The topocentric arrival times were reduced to the barycentre of the solar system with coordinates taken from the Astronomical Ephemeris (Earth-Sun) and computed from the APAE (Sun-barycentre) including the greater planets except Mercury. The times were corrected for the relativistic effects of the motion of the Earth according to Clemence⁵. The computed phase $\varphi(t)$ at the instant t is defined by

$$\varphi(t) = \varphi_0 + (t - t_0) \nu + (t - t_0)^2 \dot{\nu}/2 + (t - t_0)^3 \ddot{\nu}/6 \quad (1)$$

where the four parameters of the cubic polynomial are φ_0 , ν , $\dot{\nu}$, and $\ddot{\nu}$: the computed phase, the frequency and its two time derivatives at the instant $t = t_0$.

A phase residual O-C of each observed barycentric time of arrival t is defined by

$$O-C = \varphi(t) - N \quad (2)$$

where N is the number of the pulse in a continuous counting over the whole observing period. In order to avoid miscounting, estimates of the four parameters have been made including successive larger time spans by making $\Sigma(O-C)^2$ a minimum.

The parameters obtained by the last unweighted least squares fit of our observations are listed in Table 1

together with the corresponding values from a weighted least squares fit to a cubic polynomial plus the 77 day sine oscillation from Table 1 of Richards *et al.*². The notation in units of powers of days⁻¹ allows a simple estimate of the influence on the phase of the different parameters and their errors by multiplying them by the corresponding power of the time elapsed. The epoch of JD 2440 492.5 has been chosen because it is believed to be the approximate date of the speed-up^{6,7,9}.

Table 1. CUBIC PARAMETERS OF THE ARRIVAL TIMES AFTER THE SPEED-UP AND IN BRACKETS THE PARAMETERS BEFORE THE SPEED-UP

t_0	=	2440 492.5	Date of speed-up
		(2440 492.5)	
φ_0	=	0.5672 \pm 104	
		(0.4561 \pm 22)	
Change	+	0.1111 \pm 106	
ν	=	2610 060.283 375 \pm 401 days ⁻¹	
		(2610 060.280 542) \pm 123	
		+0.002 833 \pm 419	
$\dot{\nu}/2$	=	-1.439 658 03 \pm 461 days ⁻²	
		(-1.439 661 21 \pm 184)	
		+0.000 003 18 \pm 496	
$\ddot{\nu}/6$	=	(1.447 \pm 15) $\times 10^{-6}$ days ⁻³	
		((1.140 \pm 8) $\times 10^{-6}$)	
		(+0.307 \pm 17) $\times 10^{-6}$	
Braking parameter n	=	2.73 \pm 6	
		(2.15 \pm 3)	

Errors are formal mean errors in units of the last decimal given.

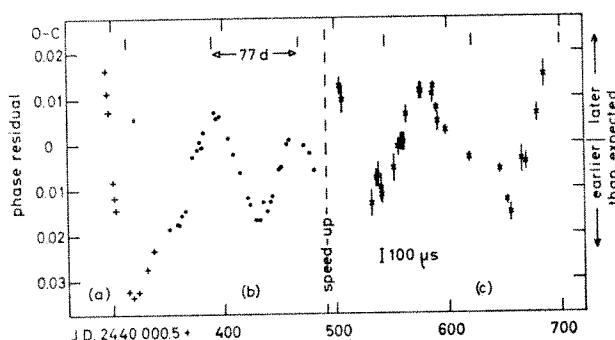


Fig. 1. Residuals for NP 0532. O-C = Observed time of pulse - expected time of pulse in units of periods. a, Optical observations of Boynton *et al.*¹ versus parameters of Richards *et al.* (see Table 1) assuming $\varphi_0 = 0.599$ instead of $\varphi_0 = 0.456$. b, Radio observations of Richards *et al.*² versus parameters as a. c, Optical observations of this paper versus cubic parameters (Table 1).

The residuals from the obtained elements (Table 1) are shown in Fig. 1c with error bars indicating the internal mean error of the value for each night. For comparison the residuals observed by Richards *et al.*² and Boynton *et al.*¹ are shown in Figs. 1b and 1a respectively, corresponding to the elements given in Table 1. Richards *et al.*² defined his residuals as $N - N_{fit}$ (private communication) which is equivalent to $N - \varphi(t) = -(O-C)$ in our notation. They therefore appear with inverted signs in our Fig. 1b. Because Boynton does not give a parameter equivalent to φ_0 , his residuals from Richards's elements have been shifted vertically (that is, in phase) to give an almost continuous function for the residuals. It is unfortunate that none of the three observing periods overlap in part and that the speed-up occurred just in the gap between the radio observations and ours. Overlaps would have removed the uncertainty in φ_0 of Boynton's measurements. The $\Delta\varphi_0 = 0.1111$ in Table 1 implies that the average frequency during the 23 days between

the last radio observation and our first observation differed by $\Delta\nu = 0.005$ days⁻¹, which is in agreement with the reported values of the speed-up^{6,7} of $\Delta\nu = 0.006$ days⁻¹.

It is obvious from Fig. 1 that the period of 77 days has not been persistent over more than the time span of 150 days of the radio observations.

From the data available until now the nature of the wobble in arrival times with a typical time scale of several months is still vague. The proposed interpretations in terms of a planetary companion^{3,10} or free precession⁹ cannot be maintained in their simple form. It is not possible to compute reliably the secular values of $\dot{\nu}$ and the braking index⁸ $n = \nu\ddot{\nu}/\dot{\nu}^2$ because $\dot{\nu}$ is strongly affected by the wobble. Furthermore, it is not known whether the wobble is associated with the speed-up. According to our data there is also the possibility that smaller speed-ups or a wobble of a time scale of about a week is present.

The method proposed by Sutherland *et al.*¹¹ for deriving the mass of the pulsar from the changes in the frequency and its first two derivatives caused by the starquake at the time of speed-up does not give a reliable value because the change in $\dot{\nu}$ is very small and relatively uncertain.

For these reasons it is important to observe NP 0532 as often and precisely as possible and to publish the absolute times of arrival in the UTC or AI scale so as to allow a comparison between different observers. It is recommended that geocentric as well as barycentric arrival times be published because it may be desirable to reduce them with changed elements of the Earth's orbit.

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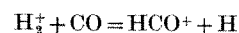
Carrier of the Interstellar 89.190 GHz Line

THE unidentified interstellar line discovered by Snyder and Buhl¹ (reported at the IAU general assembly, Brighton) with estimated rest frequency of 89.190 GHz is well within estimation error of the first rotational transition expected for the molecular ion HCO⁺. This species is isoelectronic with HCN and is extremely stable. The molecular geometry expected for this ion is linear, with bond lengths $r_{\text{CH}} = 1.06$ Å and $r_{\text{CO}} = 1.115$ Å. These distances are the CH bond length in HCN and the CO bond length in the ion CO⁺. This set of geometric parameters gives a rotational constant $B = 44.623$ GHz. The lowest transition frequency, $\nu = 2B$, is predicted at 89.246 GHz.

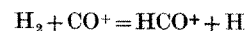
The electronic structure of HCO⁺ is ¹Σ and the only hyperfine structure expected is the hydrogen spin rotational interaction whose magnitude is of the order of 10 kHz and therefore much less than the Doppler line width. The observed line appears to be single rather than a component of a hyperfine multiplet.

Fortunately it is possible to test this present speculation. The assignment of 89.190 GHz to H¹³CO predicts that the analogous transition in H¹³CO occurs near 86.708 GHz.

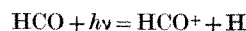
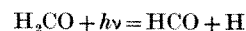
The stability of HCO⁺ is apparent from the low ionization potential of HCO, namely, 9.88 eV. (The ionization potential of C^I is 11.25 eV.) The production of interstellar HCO⁺ can proceed by several routes. The reactions



and



are observed in the laboratory to have rate constants of 10^{-9} cm³ s⁻¹. The photochemical production



can also occur. Obviously the spatial correlation of HCO⁺ (if the present assignment proves correct) with H₂CO will be of interest.

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Galactic X-Ray Background Component: the Difficulties with the Inner- Bremsstrahlung

IT has recently been claimed¹ that the inner-Bremsstrahlung associated with suprathermal particles can account for the X-radiation at 0.26 keV in the galactic plane observed by Bunner *et al.*².

The energy spectrum of the suprathermal particles used in this calculation was obtained by multiplying the observed spectrum by a demodulating factor³ to take into account the influence of the solar wind. But the self-absorption, which is important for energies of the order of 0.26 keV, was not taken into account. Moreover, the ionization rate ξ of the interstellar gas by these suprathermal particles imposes limits on the energy cut-off of the spectrum and on the particle flux.

The calculation given here shows that when the gas self-absorption and the ionization rate are included, the inner-Bremsstrahlung produces an X-ray flux which is several orders of magnitude smaller than that reported by Bunner *et al.*².

For the sake of simplicity, assume that the flux of suprathermal protons is given by

$$J_p(E) = K_p \delta(E - E_0) \text{ protons cm}^{-2} \cdot \text{s}^{-1} \cdot \text{sr}^{-1} \cdot \text{MeV}^{-1} \quad (1)$$

The final results are not sensitive to the choice of the energy E_0 .

The ionization rate produced by these suprathermal particles is

$$\xi = \frac{5}{3} y_0 \int 4\pi\sigma_H(E) J_p(E) dE \quad (2)$$

where the factor 5/3 takes into account the secondary ionization⁴, the factor $y_0 \approx 2.0$ corrects for the presence of particles other than protons in the cosmic ray chemical composition and also for the presence of 10 per cent in number of He in the interstellar gas⁵. $\sigma_H(E)$ is the cross-section for ionization of H by protons with energy E .

Substituting equations 1 and 2 into using appropriate numerical values,

$$\xi = 9.4 \times 10^{-16} \left(\frac{K_p}{E_0} \right) s^{-1} \quad (3)$$

The photon production rate per unit volume and energy interval is

$$q(\varepsilon) = 4\pi n y_0 \int_{(M/m)\varepsilon}^{\infty} \sigma_B(E, \varepsilon) J_p(E) dE \quad (4)$$

where n is the electron number density (free + bound electrons), $\sigma_B(E, \varepsilon)$ is the Bremsstrahlung cross-section and M/m is the proton to electron mass ratio.

With the flux of suprathermal protons given by equation 1, one finds from equation 4 that

$$q(\varepsilon) = 0 \text{ if } \frac{M}{m} \varepsilon > E_0 \quad (5)$$

and

$$q(\varepsilon) = 4\pi y_0 n \sigma_B(E_0, \varepsilon) K_p \text{ if } \frac{M}{m} \varepsilon < E_0 \quad (6)$$

Thus, if the flux at $\varepsilon = 0.26$ keV is to be calculated then $E_0 > 0.5$ MeV.

The photon flux, when the self-absorption by the gas is taken into account, is

$$J(\varepsilon) = \frac{q(\varepsilon)}{4\pi \kappa(\varepsilon) n_H} [1 - \exp(-\kappa(\varepsilon) N_H)] \text{ photons} \\ \text{cm}^{-2} \cdot \text{s}^{-1} \cdot \text{sr}^{-1} \cdot \text{keV}^{-1} \quad (7)$$

where
$$\kappa(\varepsilon) \approx \frac{5.07 \times 10^{-23}}{\varepsilon_{\text{keV}}^{3/2}} \text{ cm}^2$$

is the photoionization cross-section⁷, n_H is the H density and N_H is the columnar hydrogen density.

Combining equations 3, 6 and 7 and using the Bremsstrahlung cross-section given by Heitler⁸, the equation for the photon flux can be written as

$$J(\varepsilon) = 1.2 \times 10^{14} \varepsilon_{\text{keV}}^{2.2} \xi \\ \lg \left\{ \frac{1 + (1-x)^{1/2}}{x^{1/2}} \right\} \cdot [1 - \exp(-\kappa N_H)] \quad (8)$$

where
$$x = \frac{M}{m} \frac{\varepsilon}{E_0}$$

For calculating the flux at $\varepsilon = 0.26$ keV, the term which depends on E_0 is logarithmic so it is not very sensitive to E_0 , and in the calculations, E_0 was assumed to be equal to 2 MeV ($x = 0.24$). Because $\kappa(\varepsilon = 0.26 \text{ keV}) N_H > 1$

in the galactic plane, equation 8 gives

$$J(\varepsilon = 0.26 \text{ keV}) \approx 6.2 \times 10^{12} \xi \text{ photons cm}^{-2} \cdot \text{s}^{-1} \cdot \text{sr}^{-1} \cdot \text{keV}^{-1} \quad (9)$$

The upper limits estimated by several authors⁹⁻¹¹ for the ionization rate are of the order of $\xi \approx 10^{-15} \text{ s}^{-1}$. Therefore

$$J(\varepsilon = 0.26 \text{ keV}) \approx 6.2 \times 10^{-3} \text{ photons cm}^{-2} \cdot \text{s}^{-1} \cdot \text{sr}^{-1} \cdot \text{keV}^{-1} \quad (10)$$

This value is about 10^4 times smaller than the flux reported by Bunner *et al.*² and shows that it cannot be a result of inner-Bremsstrahlung.

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Solar Eclipse Measurements at 16 and 30 GHz

On March 7, 1970, the solar eclipse was observed at 30 and 16 GHz using the Crawford Hill Sun tracker¹. At Crawford Hill the eclipse was partial with greatest magnitude of 0.964. The measurement consisted of an accurate determination of the residual flux at the time of greatest magnitude as a proportion of the total solar flux. From these data, the size of the radio Sun at 30 and 16 GHz is determined.

The Sun tracker consists of a five foot by nine foot plane reflector mounted as a polar heliostat to reflect the Sun's radiation into a conical horn-reflector antenna of four foot aperture. The hour angle motion for tracking the Sun is provided by driving the reflector about its polar axis at a rate of 24 h per revolution. The output of the horn-reflector antenna is in a circular waveguide. One linear polarization is split off by a polarization coupler for the 16 GHz receiver and the orthogonal polarization passes through a waveguide taper to the 30 GHz receiver.

The receivers use balanced Schottky-barrier diode mixers with broadband input circuits allowing double sideband response and are directly connected to transistor IF preamplifiers. The double sideband noise temperatures of the receivers are 840 K at 30 GHz and 1,300 K at 16 GHz. Diode switches are used to compare the antenna temperatures, at 30 and 16 GHz, with room temperature terminations. The difference voltages are detected, amplified and presented on chart recorders.

The antenna pattern was measured at 30 GHz using a far field transmitter and the half-power beam width was found to be 35'. As the pattern was normal, the 16 GHz pattern was obtained by scaling. The radiometer was calibrated by means of matched waveguide terminations at liquid nitrogen temperature. Receiver gain was monitored several times during the measurement and was found to remain constant to within 1 per cent.

Weather conditions during the eclipse were good. The atmosphere was slightly hazy but remained stable throughout the measurement. Atmospheric extinction, measured before and after the eclipse by observing the variation of atmospheric radiation with zenith angle, was found to have remained constant, with values of 4 per cent at 16 and 7 per cent at 30 GHz.

At the time of greatest eclipse, the antenna temperature, corrected for atmospheric attenuation, was due to contributions both from the Moon and the Sun. The contribution of the Moon was known from lunar phase curves obtained, using the sun tracker, over a period of several months before the eclipse. After subtracting the Moon's contribution, the remaining antenna temperature was compared with that due to the total Sun before the eclipse, both having first been corrected for pattern weighting. The fraction of total solar flux visible at the time of the greatest eclipse was 5.6 per cent at 30 and 6.53 per cent at 16 GHz. It might be mentioned that the range of measured antenna temperatures, before and during the eclipse, was restricted, by means of precision waveguide attenuators, to regions over which the receivers had been measured to be linear to better than 1 per cent.

In order to calculate the size of the radio Sun from the measurements of residual flux, the brightness distribution at the limb of the Sun must be known. Measurements by Simon² at millimetre wavelengths indicate a flat distribution of solar limb brightness (that is, with a rectangular edge) rather than a decreasing or limb brightened distribution. This result is supported by Simon and Zirin³ who, in a recent review of solar observations, find no limb brightening and a remarkably flat brightness distribution across the disk. The eclipse curve obtained in the present measurement also showed no evidence of limb brightening and the results were consistent with a flat brightness distribution at the limb.

Assuming then a flat brightness distribution, and using the known geometry of the eclipse, the size of the radio Sun which gives the measured values of residual flux can be calculated. The values of the ratio of solar radio radius to optical radius so obtained are 1.021 at 30 GHz and 1.031 at 16 GHz. These ratios are estimated to be correct to within ± 0.005 .

These results also indicate that, at 30 and 16 GHz respectively, 4.2 and 6.2 per cent of the total solar flux come from outside the optical disk.

Although I am not aware of previous eclipse measurements at 30 GHz, recent solar observations at 34.9 GHz by Kalaghan and Telford (unpublished) indicate a radio disk size 3 ± 1 per cent larger than the optical disk, in agreement with my results. The results of Soviet observations at 18.7 GHz (quoted by Castelli and Aarons⁴) during the eclipses of 1958 and 1961 give radio disk sizes which are 3 and 4.8 per cent respectively greater than the optical disk. Although the 1961 result is not in agreement with the 16 GHz measurement of this report the 1958 result is in excellent agreement. It is interesting to note that the 1958 and 1970 eclipses took place at approximately the same point in the solar activity cycle.

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Electronic Processes in Bilayer Lipid Membranes

THE appearance of an e.m.f. on illumination of certain "black" or bilayer lipid membranes (BLM) containing chlorophylls and/or carotenoid pigments has been reported recently¹. Some of the earlier findings have been confirmed by Hesketh². The magnitude of the observed photo-e.m.f.'s was generally small, of the order of a few mV, which may be because the systems investigated have been in "symmetrical" conditions. The two aqueous solutions separated by the BLM were identical even though the exciting light approached the membrane from one direction only. This uneven illumination of the BLM and its supporting Plateau-Gibbs border by exciting light together with a slight mismatching of the contacting electrodes was apparently sufficient to give rise to the observed photo-electric effects. These light-induced effects have been explained in terms of charge carrier production and separation, as electrons and holes are generated in the BLM. It was predicted, if the above explanations were correct, that one side of the biface would become oxidizing and the other side reducing¹. We now report the results of further experiments with photoactive BLM under "asymmetrical" conditions. We have found that an open-circuit photo-e.m.f. greater than 100 mV can be generated across the BLM, and we also describe here other new findings on the photoactive BLM.

Experimental methods for the present work were essentially those of previous studies¹. We measured the photo-e.m.f. and photoelectric spectrum of BLM separating two aqueous phases, creating asymmetrical conditions across the BLM by the addition of a modifier to one side or different modifiers to the opposite sides of the bathing solutions. Because these types of BLM's studied were sensitive to H⁺ (a membrane potential of 50–58 mV per ten-fold difference of H⁺ concentration could be produced in the pH range of 4–6), they were all formed in 0.1 M acetate buffer at pH 5.0 unless otherwise noted. In the buffer medium, the introduction of modifiers to one or both sides of the bathing solutions usually generated a dark membrane voltage. The magnitude of the dark voltage varied from a few to tens of mV depending on the modifier used. In the absence of modifiers, the photo-e.m.f.'s of the BLM were small but easily measurable (Table 1). The photo-e.m.f. of the BLM could be dramatically increased by two or three orders of magnitude when the modifiers were present in the bathing solutions. Furthermore, the time constant of photoresponse was greatly reduced and the system seemed to be reversible to exciting light. The photo-effects in the presence of Fe³⁺ were independent of the direction of light but the polarity of the photo-e.m.f. was determined by the location of Fe³⁺, being always negative with respect to the iron-free side. The open-circuit photo-e.m.f. (E_{op}) as a function of light intensity (I) has been found to follow the simple equation

$$E_{op} = A \log \left(1 + \frac{I}{B} \right) \quad (1)$$

where A and B are constant for a given BLM at a particular temperature. In conditions of low light intensity ($B \gg I$), as would be expected, E_{op} was shown to be directly proportional to I . The time constants for the rise and

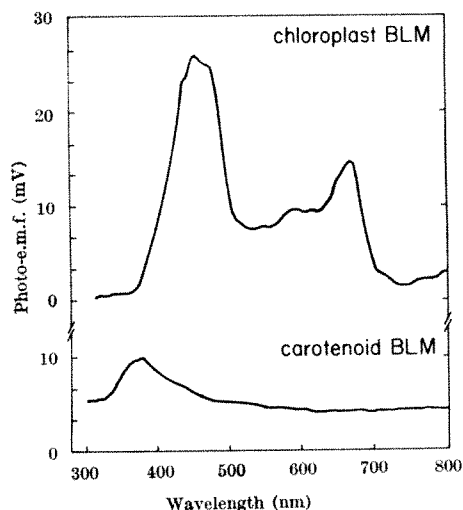


Fig. 1. Spectral sensitivity of two types of photoactive BLM's. The photoelectric spectra were obtained by exciting the BLM with light from a 1,000 W xenon lamp through a monochromator. The open-circuit photo-e.m.f. across the BLM was measured with a Cary vibrating-reed electrometer and recorded with a Keithley recorder. Both BLM's were formed in 0.1 M acetate buffer at pH 5. The outer chamber contained 10^{-3} M FeCl_3 . Chloroplast BLM was prepared from spinach extract. Carotenoid BLM was prepared from all-*trans* retinal in oxidized cholesterol-lecithin solution.

decay of the photo-e.m.f. have been found to vary from less than 50 ms to several tens of seconds, depending on the modifier present. The shape of the curves also depended on the modifier. In general, however, the presence of a reducing agent or the absence of a good electron acceptor greatly increased the time constants. In the cases investigated the photo-e.m.f. sensitivity (defined as dE_{op}/dI) was found to be inversely proportional to I . With an external load resistor (R), the power output of the BLM was evaluated as i^2R , where i is the photocurrent. The maximum power output was obtained (10^{-4} – 10^{-3} m W/cm² BLM) when the value of the load resistance was equal to the BLM resistance. Two typical photoelectric (action) spectra, one for a chloroplast BLM and one for an all-*trans* retinal BLM, are shown in Fig. 1. The chloroplast BLM exhibited red fluorescence when excited by light of shorter wavelengths. The results show that the photo-e.m.f.'s reported here are due primarily to light absorbed by the pigments in the BLM and not to the modifiers present in the bulk solutions. Certain BLM's, however, which are otherwise not photoactive, can be sensitized by certain inorganic ions³ and organic dyes⁴ dissolved in the aqueous solution. We have observed photo-effects, similar to those previously reported, in oxidized cholesterol BLM sensitized by a variety of dyes (see Table 1) such as methylene blue, methyl red, thionine, rhodamine B, methyl viologen and others. The details of dye-sensitized BLM will be reported elsewhere.

Evidence presented here (Table 1 and Fig. 1) strongly suggests that the origin of light-induced voltages in these BLM is electronic rather than thermal. In our studies, in spite of the precautions taken to prevent direct thermal heating of the BLM, the possibility still exists that light absorbed by the BLM may be converted to heat, irrespective of wavelength used. The observed changes in potential by light may therefore be a manifestation of thermal disturbances at the biface. Also, the magnitude of the light-induced voltages reported earlier for BLM's in symmetrical conditions was of the order of tenths of mV to a few mV. This lends support to the idea that thermal effects² are relevant, which, it should be pointed out, is more plausible when the measured conductivity of the membrane is taken into account. It is difficult to argue the case convincingly for open-circuit voltage measurement, however, unless the observed voltage across the BLM is a result of differential heating—a possibility that

cannot be ruled out *a priori*. An experimental test may be made by measuring the so-called "thermoelectric power". This has been carried out recently in this laboratory by Dr Nguyen Thuong Van. It has been found that the "thermoelectric power" of the chloroplast BLM is about 60 μV per degree over the range from 15–40° C. Thus it is safe to conclude that the thermal contribution, if any, toward the observed photo-e.m.f. is unimportant.

Table 1. PHOTO-E.M.F.'S OF CHLOROPLAST BLM IN 0.1 M ACETATE BUFFER AT pH 5 (UNLESS OTHERWISE NOTED) IN VARIOUS ASYMMETRICAL CONDITIONS

Left side	Modifier in solution Right side	Photo-e.m.f. (mV)	Remarks
None	None	0.1–2	In 0.1 M KCl
H^+ (3×10^{-4} M)	H^+ (2.5×10^{-4} M)	12	In 0.1 M KCl
Fe^{3+} (10^{-3} M)	None	55–60	Fast response (30–50 ms)
None	<i>p</i> -benzoquinone	7	
None	1,4 dihydroquinone (HQ)	8	Slow response (several seconds)
Fe^{3+}	HQ	117	Fast response
Fe^{3+}	<i>p</i> -chloranil	100	
None	2-hydroxy-1,4 naphthoquinone (NQ)	5	
Fe^{3+}	NQ	87	
Ce^{4+}	None	20	As a sulphate
Ce^{4+}	NQ	90	
None	Methylene blue (3×10^{-4} M)	6	Oxidized cholesterol BLM, after 30 s illumination with white light
None	Rhodamine B (4×10^{-4} M)	0.5	Same as above
None	1,4 naphthoquinone-2-sulphonic acid (5×10^{-3} M)	3	Same as above except at pH 3
None	Methyl orange (4×10^{-3} M)	1.2	Same as above, bi-phasic response

Exciting light was provided by a 150 W projection lamp filtered through an 8-cm cell containing 5 per cent CuSO_4 . Data were taken after at least 60 min equilibration following BLM formation. The results given are the maximum values after one 20 s illumination.

The observed light-induced effects in these BLM's in asymmetrical conditions can perhaps be explained best by reference to Fig. 2. The photoactive BLM is depicted as similar to that of a barrier-layer type of photovoltaic cell. In the present case a "barrier layer" is assumed to be formed on the side where electron acceptors (Fe^{3+}) are located. The incident radiant energy on the barrier-layer causes electrons to be ejected from the donor molecules (for example excited chlorophylls) to the electron acceptors, thereby effecting a reduction reaction. In the meantime, the large number of "holes" generated near the barrier-layer drifts toward the other side of the biface where electron donors are situated. It is entirely con-

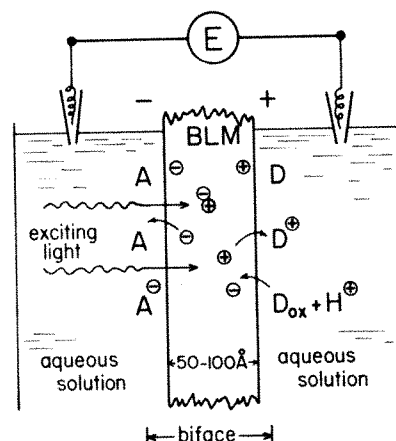
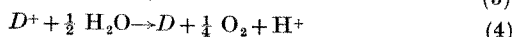
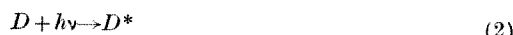


Fig. 2. Schematic illustration of the basic electronic processes in photoactive BLM separating two aqueous solutions. A, Electron acceptor; D, electron donor; D^+ and D_{ox} are oxidized forms of D; \ominus , electron; \oplus , positive hole. It is postulated that electrons and holes are generated in the BLM via exciton dissociation on illumination. See text for other details.

ceivable that in the absence of any added donor molecules water could serve as an effective donor, being oxidized in the process. The redox reactions taking place across the biface may therefore be summarized as follows



The scheme depicted in Fig. 2 is in essence a combined mode of energy and charge transfer in an ultrathin layer of poorly conducting hydrocarbon which separates two highly conducting aqueous solutions. The system is rendered photoactive by the presence of pigment molecules which serve as sites of excitation. The present scheme is reminiscent of various hypotheses put forward by Szent-Györgyi, Katz, Arnold, Calvin, Terenin and others (see refs. 1, 5) in connexion with the primary process of photosynthesis. An alternative explanation, without invoking chemical reactions at the biface, can also be suggested for the light-induced photo-e.m.f. The pigment molecules in the BLM create a potential barrier at the side of the biface containing electron acceptors. The photogenerated electrons are preferentially trapped at that interface charging it negatively. The positive holes, drifting toward the other side of the biface, make it positive in a manner analogous to that of a photovoltaic cell.

The central point in either one of the above explanations is that charge carriers in the form of electrons and holes can be generated by strongly absorbed light in an ultrathin (<100 Å) lipid membrane immersed in aqueous solution. The highly insulating layer of liquid hydrocarbon in the interior of the BLM seems to be crucial in producing the photovoltaic effect because little or no photo-response could be elicited from BLM with a resistance below $10^5 \Omega\text{-cm}^2$. Apparently, the membrane may be internally short-circuited. Other evidence supporting electronic processes that can occur in high resistance BLM's is provided by iodine-doped oxidized cholesterol BLM. Earlier, Lauger *et al.*⁶ reported that lecithin BLM could be made sensitive to iodide. We have found that a BLM can be made into an iodide-specific electrode simply by incorporating a minute quantity of iodine into the lipid solution before BLM formation. This behaviour of iodine-doped BLM is like that of a metallic electrode which can revert to its ion (for instance, Ag electrode to Ag^+). In addition, a chloroplast BLM in the dark can function like a redox electrode, just as the platinum electrode in the next experiment. In this test, a known quantity of Fe^{2+} was added to one side of the BLM; it was then titrated with KMnO_4 . A titration curve of classical form was obtained with a rapid potential rise at the equivalent concentration. The results indicate that a chloroplast BLM (or other BLM after suitable modification) can behave like a redox electrode. This, together with the results of photo-response experiments, implies that the BLM, as well as serving as a barrier separating two aqueous solutions, can act as a conductor for facilitating electron (or hole) transfer.

These findings are of interest not only in the investigation of the primary processes in photosynthesis but also for chemiosmotic theory^{7,8}, in which redox reactions across a lipid membrane are believed to be highly important.

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Age and Origin of Aeolian Sand in the Vale of York

SANDY soils in the Vale of York within the area of glacial Lake Humber and north of the Esrick moraine¹ suffered severe wind erosion during early 1967 and 1968²⁻⁴. I studied the age and character of the sands during detailed soil mapping near York.

Over Weichsel till and lacustrine deposits there are small spreads of fluvial medium to coarse sands and hummocky areas of quartzose fine sand with mounds up to 6 m high. Fig. 1, illustrating the distribution of fine sand between York and Malton and compiled from unpublished Soil Survey maps, indicates that the largest areas occur between Sutton-on-the-Forest and Sand Hutton.

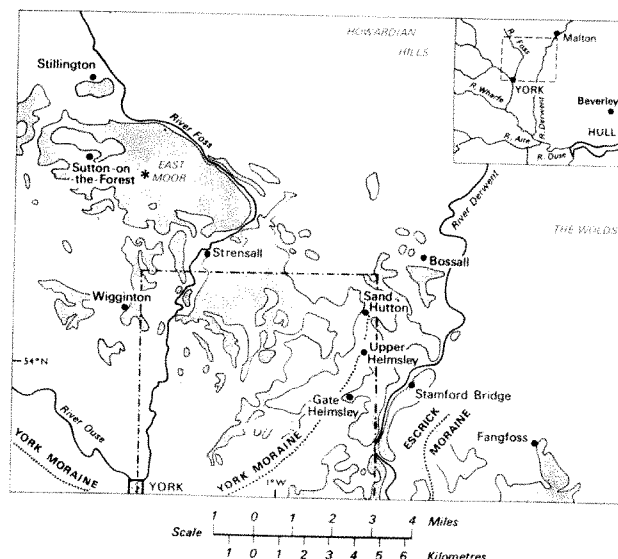


Fig. 1. Distribution of aeolian sand between York and Malton. *Site of radiocarbon-dated peat (code No. N-488; $10,700 \pm 190$ yr BP) from below the aeolian sand. Stippled areas, aeolian sand; ---, area of Fig. 3.

The fine sand has oval and subangular quartz grains with a large proportion between 0.1 and 0.15 mm in diameter (Fig. 2), the particle size distribution being determined by appropriate B.S. sieves. This is typical of aeolian sands⁵ and resembles that of sand blown on to a road at Upper Helmsley during the gales of late March 1968. From this it is inferred that the mounds are fossil dunes modified by solifluction, rainfall, stream erosion and cultivation. As most are over level lake deposits, the relief is probably wholly caused by dune formation and erosion. Many mounds are longitudinal and trend north-west/south-east and north/south; barchan-like crescentic mounds also occur. Fig. 3 shows their distribution near York. The sand was probably winnowed from coarser fluvio-glacial drift deposited when the Weichsel Vale of York ice-sheet melted. The material

is homogeneous with little or no evidence of stratification; any that existed was probably destroyed by cryoturbation and pedological processes. At a few places, however, there are thin gyttja-like layers a few centimetres thick, which could have formed in pools over permafrost; similar conditions occur at present in dune areas in Arctic Quebec⁶ where *Sphagnum* bog is over permafrost less than 23 cm below the surface.

At East Moor near Sutton-on-the-Forest there is a 3 km² raised area of aeolian sand at 18–24 m OD; it is only slightly hummocky and overlies clayey till, penetrated by more than 35 cm wedges of compacted sand resembling ice-wedge pseudomorphs and containing small ventifacts and organic remains. As ice wedges form in a permafrost environment, their presence indicates that the sand accumulated during a cold period. In one place there is a 20 cm layer of compact humified peat with woody remains at a depth of 150 to 170 cm and 30 cm above the junction of aeolian sand and till. A sample collected in 1964 from a borehole (grid ref. SE 608640) gave a radiocarbon age (laboratory No. N-488) of $10,700 \pm 190$ yr BP (5568 years half-life) or $11,000 \pm 195$ yr BP (5730 years half-life). This provides the earliest date for deposition of most of the sand, which was accumulating while the peat was forming because there are sandy layers in the upper part. The Allerød interstadial is usually dated between about 11,950 and 10,750 years BP^{7,8} so that the peat was formed then or immediately after. If formed in the Allerød, then the sand below the peat was probably deposited during the preceding cold dry tundra phase (pollen zone I) and some, if not all, of the sand above accumulated during the succeeding tundra conditions (pollen zone III). Alternatively, most of the sand could have been laid down in zone III.

Unfortunately, the original small pocket of buried peat could not be found again to obtain samples for pollen analysis, because the site was in ridge-and-furrow and partly in woodland at the time of sampling and partly cleared and levelled during 1965. The stratigraphy of a 225 cm deep pit (grid ref. SE 607640) excavated nearby is shown in Table 1.

The 1.5 cm thick frost-contorted layer of gyttja with 1 mm thick clayey and humose layers at 94 cm has a radiocarbon age (laboratory No. N-820) of 9950 ± 180 yr BP (5568 years half-life) or $10,200 \pm 190$ yr BP (5730 years half-life) and provides a limiting date for deposition of the sand below. As the original aeolian depositional phase would have ceased with the growth of forest in Boreal

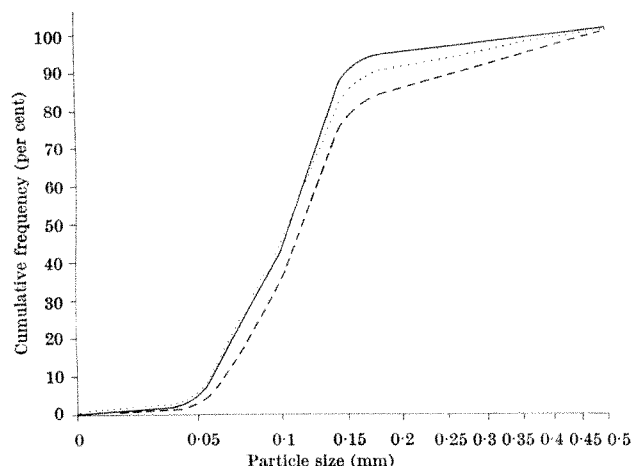


Fig. 2. Particle size analysis of soils from Late Quaternary aeolian sand and of sand blown near York in March 1968. —, Wind-blown sand deposited on road at Upper Helmsley in March 1968; ----, Bg horizon of non-calcareous gley soil at East Moor, Sutton-on-the-Forest; B/C (g) horizon of podzol at Swincarr Plantation, Upper Helmsley.

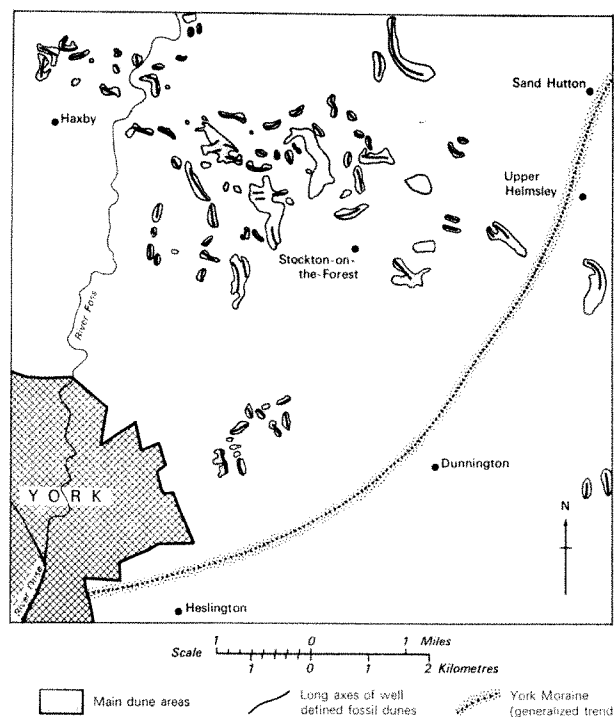


Fig. 3. Trend of Late Quaternary dunes near York.

times (after about 9,600 years BP), much of the sand above the gyttja probably accumulated after Anglo-Saxon and mediaeval forest clearance.

The gyttja does not contain pollen grains, possibly because they were decomposed by alkaline drainage water percolating from calcareous clayey marl spread in the nineteenth century to stop blowing. Table 2 lists the crumpled and eroded pollen in organic remains at 210 cm. The percentages are of total pollen of each type, excluding spores.

Table 1	
Depth (cm)	Lithology
0–94	Yellowish brown and greyish brown aeolian fine sand
94–95.5	Contorted humose clay loam (gyttja) (9950 ± 180 yr BP)
95.5–167	Greyish brown aeolian fine sand
167–225	Greyish brown and grey aeolian fine sand with discontinuous organic layers
225 +	Reddish brown clayey till chiefly from Keuper Marl

Table 2. PERCENTAGES OF TOTAL POLLEN AT 210 CM EAST MOOR

Cyperaceae	69
Gramineae	17
<i>Betula</i>	8
<i>Salix</i>	1
Ericoid	1
<i>Senecio</i> type	1
<i>Taraxacum</i>	1
<i>Ranunculus</i>	1
<i>Galium</i>	1
Total spores { <i>Selaginella</i>	12
{ Filicales	2

These species indicate a mainly treeless and open vegetation of Late-glacial (Weichselian) age, probably pollen zones I or III. Although *Selaginella* was present in late-glacial time in the British lowlands, it is significant that its spores are rarely abundant and at present it is common only on mountains and sand dunes.

The evidence suggests that much of the aeolian sand was originally deposited between about 10,700 and 9,950 years BP, that is during the later part of the Weichselian glacial stage and early Flandrian (Post-glacial) time when vegetation was sparse and the ground partly frozen. It is largely these areas of sand, and some of morainic fine sands with ventifacts, that now blow.

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Preliminary Palaeomagnetic Data from the Plio-Pleistocene of Jamaica

CORRELATIONS, using deep sea cores, between the geomagnetic time scale¹⁻³ and the planktonic foraminiferal biostratigraphy of the later Tertiary and Quaternary have received much attention in the past few years⁴⁻⁹, but only a few studies of other Quaternary sediments have yet been published. Montfrans and Hospers¹⁰ have given an account of progress made in Pleistocene continental strata of the Netherlands, but there seems to have been little attempt to relate the recently developed Plio-Pleistocene planktonic foraminiferal biostratigraphy to the geomagnetic time scale in land-based sections, although there should be several advantages of sampling such sections. For example, the initial orientation of each sample can be determined with precision; field inspection of the collecting site can ascertain the nature of the structural or stratigraphical complications; sampling can often be carried out repeatedly at any required interval; and samples for palaeontological analysis can also be collected. In general, land-based sections are considerably more expanded than deep sea cores and so might be expected to yield more reliable data on geomagnetic events of short duration. An accurate appraisal of the geomagnetic stratigraphy beyond the past 3 million years or so should therefore be effectively developed using land-based sequences.

We therefore collected fourteen orientated samples from the Plio-Pleistocene of the Coastal Group, Jamaica, West Indies^{10,12} (Fig. 1). The samples came from fresh exposures and showed no adverse effects of post-depositional alteration such as weathering, and generally compared well in quality with those recovered from deep sea cores.

Polarity transitions were detected by 180° reversals in declination following demagnetization at 150 oersteds to remove unstable components. Direction and intensity of magnetization was measured using a five H₃ spinner magnetometer, with the X, Y and Z axes each measured four separate times to eliminate or minimize inhomogeneities. The results were processed by computer (Table 1).

Bulk samples for palaeontological data were collected with each geomagnetic sample. The combined analytical results are shown in Fig. 2. Samples ER 1114 to 1122 were obtained from a single undisturbed section exposed on the north side of Innes Bay (Fig. 1). Samples previously obtained here^{13,14} indicated the presence of zones N19, N20 and N21 of Banner and Blow¹⁵. Our sequence

Table 1. JAMAICAN GEOMAGNETIC DATA, FOLLOWING DEMAGNETIZATION TREATMENT OF 150 OERSTEDS

Sample number	Declination (D, degrees)	Inclination (I, degrees)	Intensity (J, e.m.u. g ⁻¹)	Suggested polarity
ER-1076	85.0	84.1	2.140E-07	N
ER-1110	275.7	65.7	1.535E-07	T
ER-1111	314.6	65.4	1.939E-07	N
ER-1114	342.8	29.8	3.927E-07	N
ER-1115	228.7	9.1	1.595E-07	R
ER-1116	0.6	21.4	7.341E-07	N
ER-1117	2.4	31.4	6.726E-07	N
ER-1118	130.3	66.1	1.321E-06	T
ER-1119	100.1	81.6	7.514E-07	T
ER-1120	76.2	69.9	1.116E-06	N
ER-1121	188.3	34.0	5.938E-07	R
ER-1122	256.3	47.1	3.525E-07	T
ER-1123	267.3	19.1	5.938E-07	T
ER-1146	342.0	53.4	6.369E-07	N

E-07 is computer expression of negative exponent, that is $\times 10^{-7}$. N = Normal; R = reversed; T = transitional.

shows the overlap in the ranges of *Globorotalia tosaensis* and *Globoquadrina altispira* noted previously by other workers^{7,9,16}.

Sample ER 1146 was collected at the classic Bowden molluscan locality from a marl layer in the highest part of the shell beds. Originally referred to the late middle Miocene by Woodring¹⁷, this locality has also been placed in the Pliocene^{11,15} and earliest Pleistocene^{9,18}.

Sample ER 1076 was collected from near the base of Navy Island Member of the Manchioneal Formation, considered to have been deposited during the Aftonian interglacial interval of the Pleistocene¹². Sample ER 1076 contains a cool-water fauna of the lowermost transgressive Manchioneal Formation, with common *Globorotalia inflata*. Up section this changes quickly to a warm-water fauna with common left-coiled *G. menardii* more typical of the Aftonian. This horizon seems to be transitional between the cold Nebraskan and warm Aftonian stages.

Comparison of the geomagnetic events with the planktonic foraminiferal succession of Fig. 2 permits comparison with the geomagnetic-planktonic datums described by Hays *et al.*⁷ and Lamb⁹. Namely, the evolutionary appearance of *Sphaeroidinella dehiscens* occurs near the Mammoth event at about 3.0 m.y. ago (not far below sample ER 1114), the extinction horizon of *Globoquadrina altispira* falls near the Kaena event at about 2.8 m.y. ago (samples ER 1121, 1110), and the first local appear-

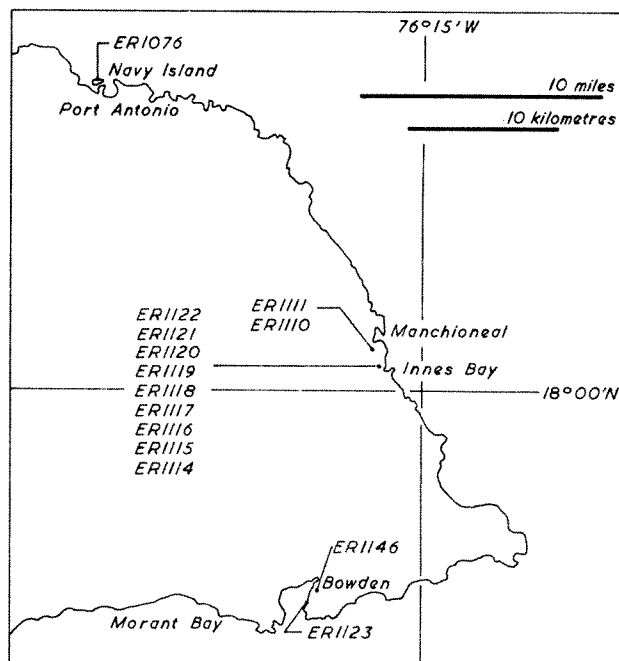


Fig. 1. Eastern Jamaica, showing locations of samples used in the study. Samples at each location are listed in stratigraphical order. (The geology of these locations has been discussed by Robinson¹⁴.)

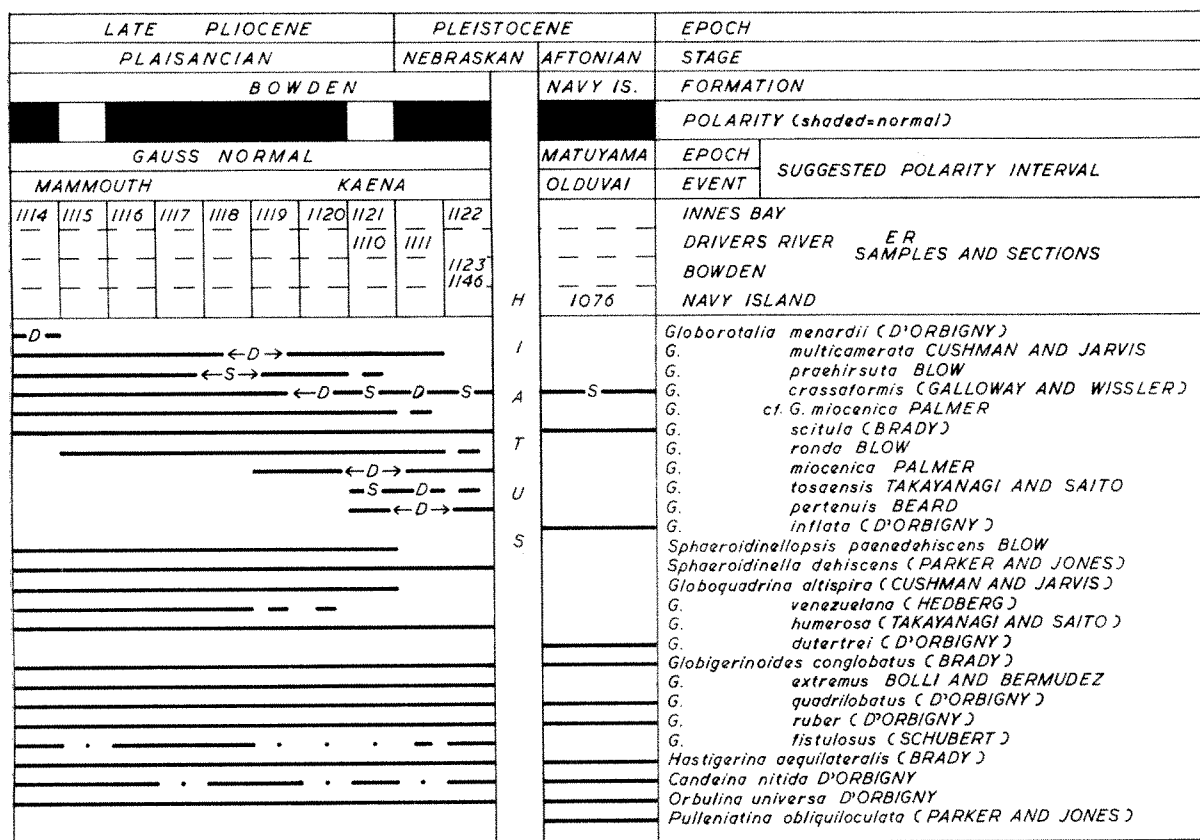


Fig. 2. Planktonic foraminiferal distribution and geomagnetic character of samples from the Upper Bowden Formation and Navy Island Member of the Manchiene Formation, Jamaica, West Indies. Coiling directions, D=dextral, S=sinistral. For each species in a sample, continuous line = common, dashed line = rare, dot = absent. Plio-Pleistocene boundary after Lamb⁹; geomagnetic polarity scale after Cox⁸.

ance of *Pulleniatina obliquiloculata* in this region occurs near the extinction horizon of *Globorotalia miocenica* near the upper Olduvai event (of Lamb⁹, not Hays *et al.*⁷) at about 2.0 m.y. ago (sample ER 1076).

These results, based on a small number of magnetic samples, show good agreement with data from deep-sea cores, and demonstrate the feasibility of supplementing and amplifying such data from land-based sections. A more detailed study of the geomagnetic stratigraphy of the Jamaican Coastal Group is in progress.

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BIOLOGICAL SCIENCES

Solvent Effect on the Conformation of Actinomycin D

THERE have been several different approaches to the stereochemistry of the interaction of actinomycin and DNA, including studies of the interaction of DNA-like polynucleotides with actinomycin^{1,2}, and of DNA with model compounds related to actinomycin³⁻⁵, using various experimental techniques. Reports on the optical rotatory dispersion (ORD) and circular dichroism (CD) data for the actinomycin-DNA complex and for actinomycin alone⁶⁻⁹, as well as unpublished results from this laboratory, indicate that the rotational strength of the optically active transitions—which can be ascribed to the dimethylaminophenoxazone chromophore of actinomycin—in the visible region of the spectrum increases in the presence of DNA. The molecular mechanism of the interaction of actinomycin with DNA is still obscure, however, for the increase in optical activity could result from a change in the conformation of actinomycin in the presence of DNA and/or from the perturbing influence of the asymmetric environment (DNA) on the chromophore transitions of actinomycin.

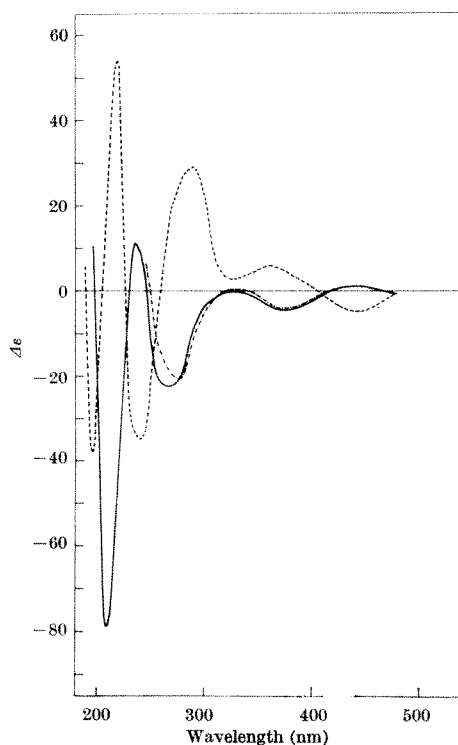


Fig. 1. Circular dichroism spectra of actinomycin D in HFA (—), CH₃CN (···), CHCl₃ (— · —).

To distinguish between these two contributions, we have studied the conformation of actinomycin D (a gift from Merck, Sharp and Dohme) in the absence of DNA. The CD spectra of actinomycin have been observed in a number of solvents of different polarity and structure. Fig. 1 records the CD spectra of actinomycin in acetonitrile, chloroform and hexafluoroacetone hydrate (HFA). An inversion of the sign of the Cotton effects is evident going from CH₃CN or CHCl₃ to HFA for all the optically active transitions in the wavelength region examined (190–500 nm). This dramatic effect, which is completely reversible, cannot result from ordinary solvent effects, to which minor differences observed between the CD spectra in acetonitrile, chloroform, ethanol⁶ and benzene⁶ can be ascribed. Furthermore, these spectra did not exhibit concentration dependence in the range of concentration and in the organic solvents used in these experiments, so that labile association is ruled out. The actinomycin chromophore may be considered to be an inherently symmetric chromophore, which is asymmetrically perturbed by the dissymmetric environment (the cyclopeptide lactone rings) but it is also an inherently dissymmetric chromophore¹⁰, having chirality.

The inversion of the CD spectra observed in different solvents and the large values of the rotational strengths, characteristic of the second type of chromophore, strongly indicate the existence of two opposite chiralities for the molecule in different solvents, which give rise to two different conformations (atropisomers) schematically represented in Fig. 2. Nothing can be said at this stage on the conformation of the peptide lactone rings, but because all the available evidence on the simple chromophore indicate that the sign of the Cotton effect is determined largely by its immediate environment, the main contribution to the rotational strength may be considered to arise from the chirality relative to the rotation of the peptide groups about the C–C bonds connecting them to the phenoxazone ring (see Fig. 2). These two conformations may be considered as two local optical antipodes, which account or the close antisymmetry of the CD spectra.

The inversion of the CD spectra and the relatively high rotational strength of the optically active transitions of the phenoxazone chromophore indicate that the molecule must be considered to be conformationally rigid in the solvents studied. This rigidity can arise from “inter-cycle” hydrophobic interactions and/or “inter-cycle” hydrogen bonds. Alternatively the effect may derive from dimerization of the molecule.

In Fig. 3 the CD spectra of actinomycin in trifluoroethanol (TFE) are reported. The CD spectrum, in spite of the normal solvent effect, can be analysed in terms of a weighed summation of the spectra obtained in CH₃CN and HFA, as can be seen in the same figure. Thus in TFE both conformations should be present. An alternative explanation, however, may be the possible existence of two opposite atropisomers in the same mole-

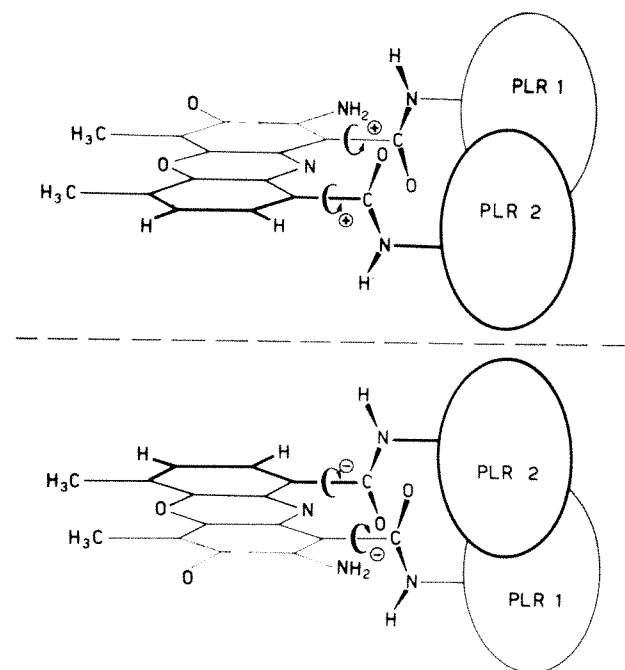


Fig. 2. Schematic drawing of the two “atropisomers” of actinomycin. PLR 1 and PLR 2 are representatives of the two peptide lactone rigs. + and - signs indicate the chirality sense.

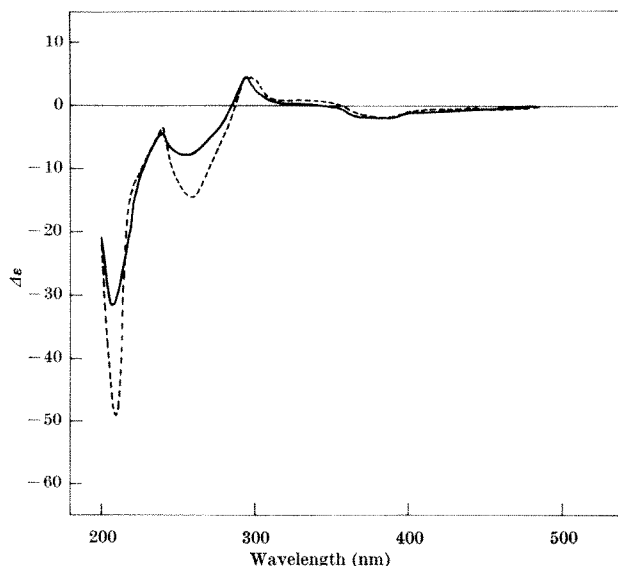


Fig. 3. Circular dichroism spectrum of actinomycin D in TFE (—). The dotted line represents the weighed summation of the circular dichroism spectra in CH₃CN (70 per cent) and HFA (30 per cent).

cule, which give rise to a local "quasi" mesoform. Because interconversion between the two atropisomers is induced by solvents, irrespective of their polarities, a specific interaction between the solvent and actinomycin may be assumed. Work is in progress with other solvents and with other techniques (NMR) to understand how this interaction takes place.

Our results indicate that different conformations of actinomycin are present in different solvents; this is also relevant with respect to interactions with DNA. The opposite helicity of the two atropisomers may be very critical for the stereospecificity of interaction with DNA. Experiments with model compounds of actinomycin and modified DNA bases are in progress to elucidate this point.

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Conformation of Actinomycin-D

NUCLEAR magnetic resonance (NMR) spectroscopy has recently been found useful for investigating the conformations of polypeptides like gramicidin S^{1,2}, valinomycin³ and so on. We now report preliminary conclusions on the conformation of the two peptide lactone rings of actinomycin D⁴ on the basis of NMR results and theoretical conformational analysis. Fig. 1 depicts the NMR spectra of actinomycin D obtained in different experimental conditions. The assignment has been made on the basis of chemical shift values, and the characteristics of the signals in double and triple resonance experiments. By examining the results obtained, some interesting features can be derived.

The spectra in Fig. 1 demonstrate that the chemically equivalent protons in the amino-acid residues are not magnetically equivalent: this is particularly evident for the NH protons of the threonine and valine residues. We might interpret this in terms of the existence of two non-equivalent peptide lactone rings in the same molecule, or in terms of the existence of two different conformations of the molecule as a whole, but with the two rings conformationally equivalent in the same molecule. The ratios between the different resonances of the chemically equivalent protons, the invariance of this relative ratio in different conditions (solvent and temperature) and the absence of this effect for the proton of the chromophoric group, however, exclude the second hypothesis.

On addition of ²H₂O, the peak at 6.2 p.p.m. corresponding to the NH₂ protons and the peak of 7.78 p.p.m.

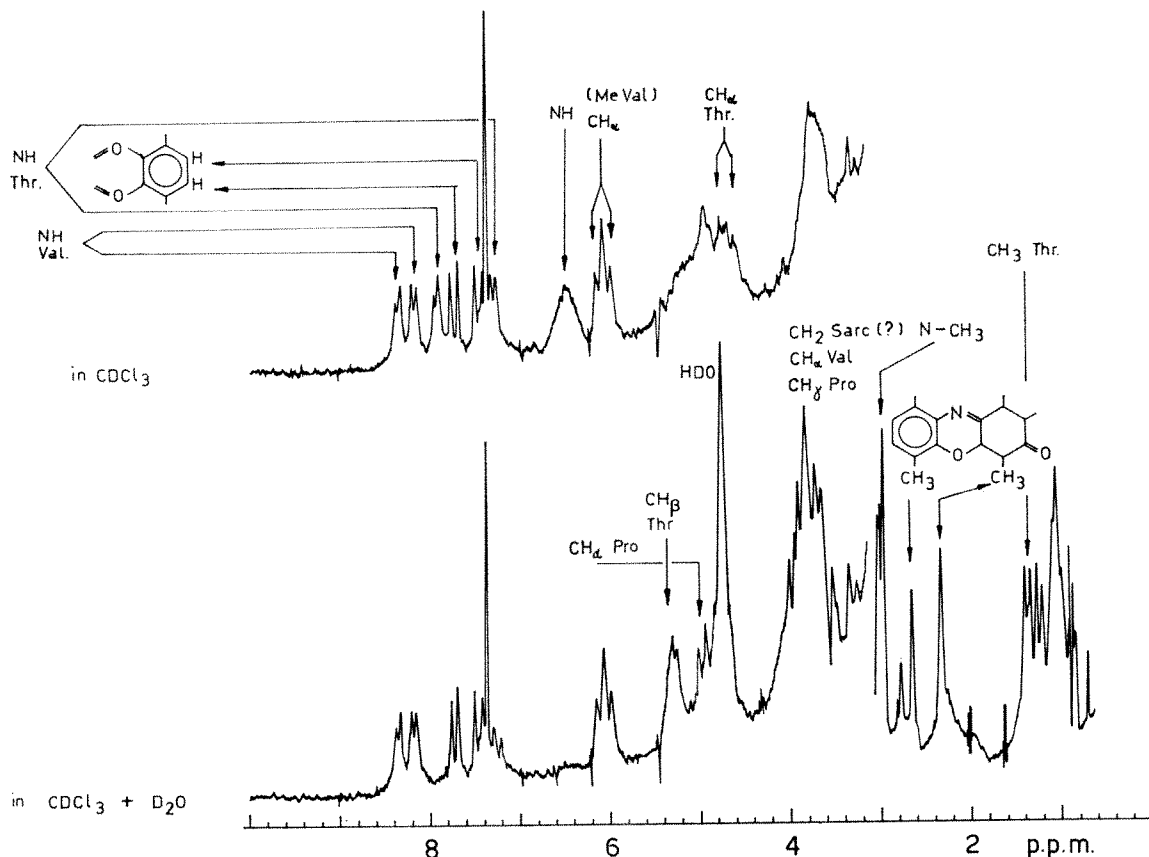


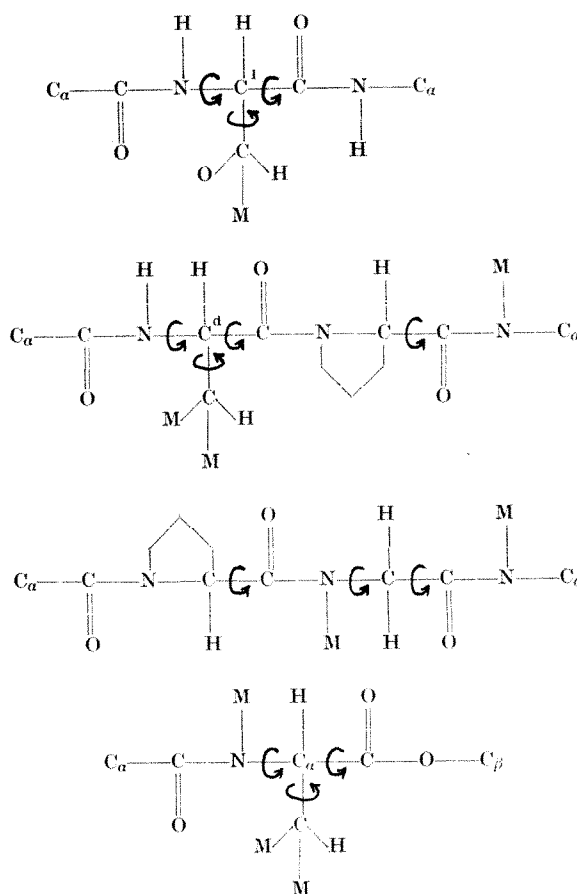
Fig. 1. NMR spectra of actinomycin D.

corresponding to threonine disappear, but the peak at 7.17 p.p.m. decreases by about one-half and the peaks at 8.27 p.p.m. and 8.07 p.p.m. corresponding to the valine residues remain. No changes are observed in the spin-spin coupling between the α -CH and the NH protons. After 3 days, only the peaks at 8.17 p.p.m. and 8.00 p.p.m. corresponding to the D-valine residues were still present. They disappear only on addition of traces of ^2HCl . These results are similar to those previously obtained in different solvents⁵ and can be explained by the presence of two strong hydrogen bonds involving the NH protons of the D-valine residues. The longer time required for the complete exchange of the NH peak at 7.17 p.p.m. corresponding to a threonine residue may suggest the presence of a weaker hydrogen bond.

In $^2\text{HCl}_3$ the N-CH₃ groups exhibit different resonance signals at 2.88 and 2.94 p.p.m. A correlation between the chemical shifts of the N-CH₃ groups and the *cis* and *trans* conformation around the peptide bond has been shown previously for polysarcosine⁶, poly-N-methyl-L-alanine^{7,8} and model compounds. In the case of actinomycin the values of the chemical shifts suggest a *trans* conformation for all the N-CH₃ protons.

A *trans* conformation of the peptide bond between the L-proline and the D-valine is also suggested by the chemical shift values of the α -CH protons of the L-proline residues (4.88), according to the previous NMR results⁹. This last point should, however, be examined carefully.

Assuming a *trans* conformation for the N-substituted peptide groups, we have attempted a conformational calculation for the peptide lactone ring. Having fixed *a priori* the geometry of the peptide and ester groups, the values of the rotation angles around the bonds which define the skeleton of the peptide lactone ring are restricted by the ring closure condition. The problem may be analysed in three steps: first, we evaluate the local conformational energy corresponding to the following unit: Second, we select the sequences of rotation angles corresponding to the local conformation minima which geometrically favour ring closure. Third, these choices are further improved by a "refining process" which allows for correct ring closure and also minimizes the conformational energy of the peptide lactone ring as a whole.



The sterically allowed conformations derived from these calculations are represented in Fig. 2. The only sterically allowed conformation of the L-proline residue corresponds to the local conformation in poly-L-proline II. This supports the conclusion already drawn about the α -CH chemical shift values of the L-proline residues. On the basis of the results of conformational analysis

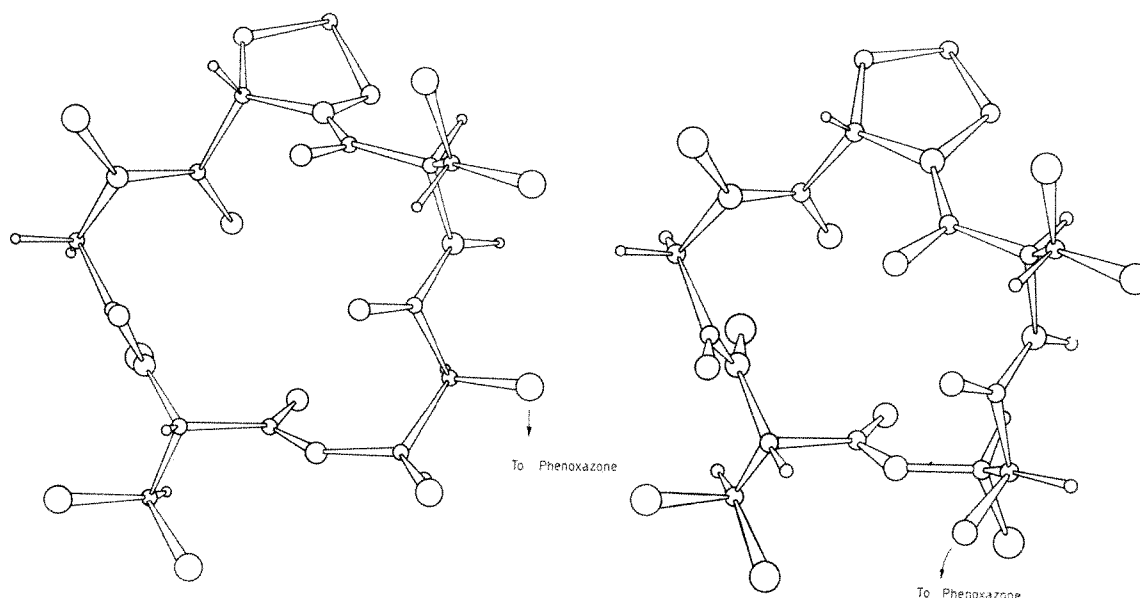


Fig. 2. Allowed conformations of peptide lactone ring.

the possible involvement of the hydrogen bond in the same cyclic chain should be ruled out, because of the steric hindrance between the N-CH₃ group and the side chain of the N-methyl-L-valine. As a consequence, the hydrogen bond detected by NMR experiments must be either between the rings of the same molecule or between rings of different molecules. The latter hypothesis can be excluded, however, because no association in non-polar solvents¹⁰ is indicated, either by the NMR exchange experiments with ²H₂O or by the smallness of the temperature effects. The ϕ angles relative to the D-valine and L-threonine residues in both conformations correspond well with the experimental values of the coupling constants between NH and the α -CH protons, on the basis of the suggestion by Bystrov *et al.*¹¹

In the models described, we would also point out that the rotation angle between the α -CH and β -CH relative to L-threonine is about 60°, in good agreement with the small value found for the coupling constant between these protons ($J=4\text{H}_2$). On the basis of previous considerations, a model of actinomycin D may be constructed (Fig. 3) which connects the two peptide lactone rings (A) at the chromophoric group, and demonstrates the possibility of two hydrogen bonds between the NH of the D-valine residues and the CO of the N-methylvaline residues. The differences between the two cyclic chains could be ascribed in this case to the non-equivalence of the conformations at the L-threonine because of the different attachment of the two rings to the chromophoric group. This supports the non-existence of a strictly dyad axis between the two rings. Moreover, X-ray results¹² support the existence of a (quasi) binary axis in the molecule. In this connexion it seems likely that one L-threonine NH group may be hydrogen bonded to the N atom of the chromophoric group. This can be correlated with the large non-equivalence between the two NH protons of the L-threonine residues.

These preliminary results seem to suggest therefore that the two cyclic chains in actinomycin are very similar, but not related by a strictly dyad axis because of the different attachment to the phenoxazone ring.

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Weak Immunological Cross-reaction between Bovine α -Lactalbumin and Hen's Egg-white Lysozyme

BOVINE α -lactalbumin and hen's egg-white lysozyme have homologous sequences¹ and similar three-dimensional structures^{2,3}. The immunological cross-reactivity between these proteins was investigated recently⁴ and no cross-reaction was detected by quantitative precipitin analysis. Our anti-lysozyme antisera, obtained as previously described⁵, also failed to precipitate α -lactalbumin. By contrast with the work of Atassi *et al.*⁴, however, it was observed that α -lactalbumin inhibits the precipitation of lysozyme by anti-lysozyme antibodies from the rabbit and the goat serum used in this study. The percentage inhibition was low (5 per cent) but reproducible and specific, for no inhibition was observed with ribonuclease.

Red blood cells were sensitized to anti-lysozyme antibodies by coupling of lysozyme or lactalbumin using *bis*-diazobenzidine⁶. Haemagglutination of the cells covered by lactalbumin in the presence of anti-lysozyme antibodies strongly suggests the existence of similar antigenic determinants on the homologous proteins.

Gel filtration on 'Sephadex G-200' of a mixture of antibodies and α -¹³¹I lactalbumin (Fig. 1) confirmed the cross-reaction of these two proteins. Similar results were obtained with anti-lactalbumin antibodies and ¹³¹I-lysozyme whereas there was no indication of binding of ¹³¹I-ribonuclease to either type of antibodies. Only 1 per cent of the heterologous antibodies seemed to bind the radioactive proteins, so that only the complexes formed with antibodies of high affinity seem to remain associated during the gel filtration.

According to a recent review⁷, one would expect that for residues implicated in antigenic determinants and thus located in exposed parts of the proteins, permitted mutations should be maximum because they are of minor importance in maintaining three-dimensional conformation. Moreover, one can assume that those parts of the surface which are kept constant during evolution, both in bovine α -lactalbumin and in hen's egg-white lysozyme, will also be maintained either in rabbit and goat lysozyme or in rabbit and goat α -lactalbumin and will not therefore evoke antibodies in these animals. Thus we conclude that studies of immunological cross-reactions between proteins will in general overlook many homologies and give a biased picture.

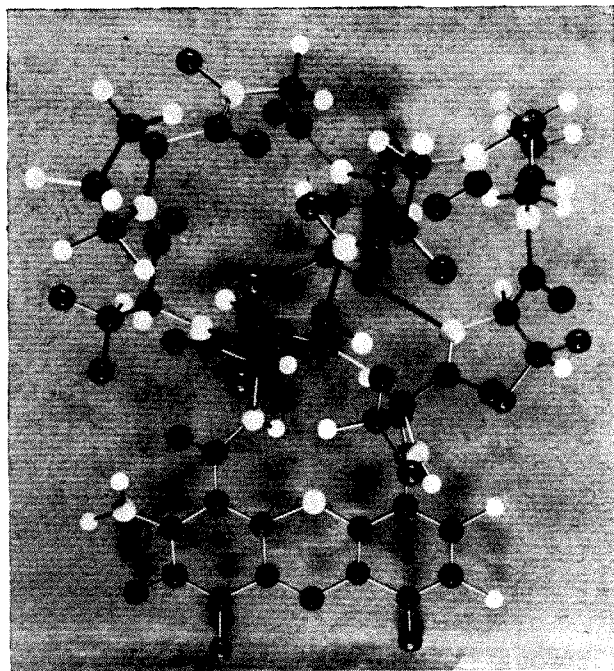


Fig. 3. Model of actinomycin D.

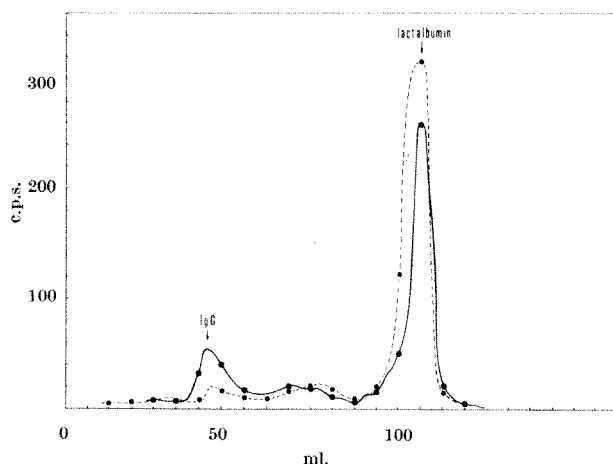


Fig. 1. Gel filtration on a 'Sephadex G-200' column (1 cm \times 100 cm) of ^{125}I -labelled α -lactalbumin mixed with anti-lysozyme rabbit serum (—) or with serum of a non-immunized rabbit (---). The elution positions of IgG and α -lactalbumin are indicated by the arrows. The samples contained 0.04 mg lactalbumin + 2.00 mg antibody or normal immunoglobulins.

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Increased Incorporation of ^{14}C -Uridine into Rat Brain RNA as a Result of Novel Electroshock

MILD electroshock is used as an aversive stimulus in many kinds of learning experiments with rats and mice. Some workers have correlated changes in brain RNA concentration with learning¹⁻³ so it seemed of interest to isolate, as well as possible, the elements in a learning situation and to determine their contribution to the observed changes in RNA.

Previous reports of the effect of electroshock on brain RNA fall into three categories: those finding a decrease⁴, an increase⁵ and no change⁶. Because of these varied findings, probably resulting from the difference in experimental conditions and levels of electroshock used, we decided to use footshock to rats at levels which we have used before as an aversive stimulus in avoidance training. To heighten the probability of obtaining measurable changes in RNA, the rats were exposed to 30 min of intermittent shock, on a schedule of 0.75 s on and 0.75 s off, delivered through a grid floor of two Lehigh Valley Electronic Chambers, 7 $\frac{3}{8}$ \times 12 \times 8 inches. The shock source was a LVE 1311 scrambler shocker consisting of a step-up

isolation transformer adjusted so that the voltage in its secondary coil was 600 V alternating current, a 100 k Ω shunt across the secondary, a 680 Ω resistance in series with it, an oil immersion grid, parallel wire grid cable and grid.

The rate of synthesis of brain RNA was compared in three groups of rats: those being shocked for the first time, those being shocked after having been shocked on the previous day, and non-shocked controls.

Thirty naive female Long-Evans hooded rats, weighing 220–280 g, were divided into three groups of ten animals each. Animals were run in sets of three, one rat from each group in each replication. All rats were kept for 2 days in quiet, dark isolation cages, designated home cages, before the first experimental day. In each set of three, one rat designated SS (shock on day 1 and shock on day 2) was subjected to foot shock for 30 min on day 1. The other two rats (QS (quiet on day 1 and shock on day 2) and QQ (quiet on day 1 and quiet on day 2)) were taken from their home cages and held in temporary isolation cages in the room in which the SS rat was being shocked. Twenty-four hours later, on day 2, all three rats were injected with 10 μl . of ^{14}C -uridine through 'Lucite' needle guides implanted over the ventricle 2 or more days earlier. This procedure of injecting into the ventricles of awake animals under hand-held gentle restraint was used both because it allows behavioural experimentation almost immediately after injection and because anaesthetics strongly affect RNA turnover in brain homogenates *in vitro* (unpublished results by F. T. G. and D. X. Freedman) and may exert similar effects *in vivo*.

Twenty minutes after injection, the SS rat and the QS rat were placed in separate compartments of the shock apparatus. The QQ rat was placed in a quiet isolation cage as on day 1, and the SS and QS rats were then given intermittent shocks for 30 min.

The three rats were decapitated immediately after this period and the brains (cerebrum minus olfactory lobes and brain stem) were removed and washed in iced KCl solution to remove surface blood and retard enzyme action. The chilled brains were blotted and sectioned into three parts, the frontal cortex (the approximate anterior half of the dorsal cortex), the rear cortex (the approximate posterior half of the dorsal cortex) and the remaining cortical and subcortical material called by us the basal brain. Each section was frozen on solid CO_2 and weighed.

The sections were homogenized separately at a concentration of 10 per cent by weight in an iced solution containing 0.15 M KCl, 0.01 M Tris and 0.5 M magnesium acetate (pH 7.4). Aliquots of 0.1 ml. were pipetted to filter disks mounted on pins. One set of disks was dried in air before scintillation counting in order to determine the total precursor radioactivity available to the brain section being analysed and an additional set of disks was run through the procedure of Schneider⁷ for RNA, as adapted to use of filter disks by Bollum⁸.

Table 1 shows the incorporation of ^{14}C -uridine into RNA expressed as the percentage of total precursor available in each of the brain sections of rats undergoing the three treatment schedules. The group of rats which received shocks only on day 2 (QS) showed significantly increased RNA in the basal brain section as compared with quiet controls (QQ) and rats shocked on both days (SS). There were no significant differences in either of the dorsal cortex sections between any of the treatment groups. In the brain taken as a whole (weighted total), the QS rats showed significantly raised levels of incorporation of radioactivity into RNA compared with the two other groups. Thus an RNA stimulating effect is found in the basal brain section but not the dorsal cortexes of rats undergoing novel electric shock. It is not found in rats which also received shocks 1 day before the testing day. We interpret this requirement for novelty as indicating that the brain RNA stimulation is likely to result from a stress response to

Table 1. INCORPORATION OF ^{14}C URIDINE INTO RNA EXPRESSED AS THE PERCENTAGE OF TOTAL PRECURSOR AVAILABLE TO BRAIN SECTIONS

Brain section	Quiet day 1	Quiet day 1	Shocked day 1
	Quiet day 2 (QQ)	Shocked day 2 (QS)	Shocked day 2 (SS)
	Per cent incorporation \pm s.e.	Per cent incorporation \pm s.e.	Per cent incorporation \pm s.e.
Frontal cortex	15.27 \pm 1.41	12.32 \pm 0.93	12.45 \pm 0.94
Rear cortex	14.32 \pm 0.63	16.85 \pm 1.32	12.57 \pm 1.72
Basal brain	14.72 \pm 0.72	18.94 \pm 0.71	16.53 \pm 0.73
Weighted total	14.77 \pm 0.66	17.32 \pm 0.75	15.12 \pm 0.70

Treatment groups (QQ-QS, QQ-SS and QS-SS) were compared for each of the different brain sections and the weighted totals. Using Student's *t* test, the following were found to be significant:

Frontal cortex	None
Rear cortex	None
Basal brain	QS-QQ; $t=3.9669$, $P<0.001$
	QS-SS; $t=2.2464$, $P<0.05$
Weighted totals	QS-QQ; $t=2.4297$, $P<0.05$

The weighted totals were calculated by determining the percentage of the total weight of the brain contributed by each section and multiplying the incorporation value of the sections by their percentage contribution and adding the total.

shock which is reduced if the animals have become habituated to the electroshock by exposure to it on the preceding day.

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Unique Glycoprotein from Mouse Milk containing the Mammary Tumour Agent

MILK from mice containing the mammary tumour agent contains a unique chemical determinant which is antigenic in rabbits¹⁻⁴. Agent-free mice can produce this antigen when they either suckle or receive by injection the milk containing the agent. We undertook the isolation and identification of this antigen to obtain insight into the mechanism of the malignant transformation—for its distribution and that of the tumorigenic potential in milk are similar⁴—and describe here our procedures.

The antigen was identified and titred by immunodiffusion using rabbit antiserum prepared against skimmed milk

containing the agent (A⁺) which was absorbed with agent-free (A⁻) skimmed milk^{4,5}. The cream was separated from whole milk by centrifugation at 800g for 40 min at 20° C. Whey was obtained from skimmed milk by precipitation with a final concentration of 0.17 M CaCl₂. The calcium caseinate micells were sedimented with a mean centrifugal force of 90,000g for 90 min⁶. The whey was dialysed overnight by repeated changes against distilled water at 6° C and the proteins from the whey were precipitated by the slow addition of neutralized saturated ammonium sulphate. The precipitate formed overnight from 30 per cent salt saturation of the whey was removed by centrifugation at 8,000g for 20 min at 6° C. The salt in the supernatant fluid was raised to 60 per cent saturation and the precipitate formed overnight was separated as before. The precipitates were resuspended in distilled water, dialysed repeatedly against distilled water and freeze-dried. A measured amount of sample was dissolved and protein content was determined⁷. Another aliquot was assayed by the Ouchterlony double diffusion procedure in 2 per cent agar with wells 5 mm apart using 4 μ l. of antiserum in the centre well and 4 μ l. of the preparation to be titred in the peripheral wells. The 30–60 per cent ammonium sulphate precipitate was further purified on ECTEOLA (epichlorhydrin triethanolamine) cellulose chromatographic columns (1.2 \times 35 cm) equilibrated at 8° C with 0.005 M phosphate buffer (pH 8.5). The elution was carried out with pH and salt gradients (pH 8.5–5.8, 0.005–0.1 M phosphate buffer). The antigen was located by immunodiffusion titration and its heterogeneity determined by analytical acrylamide disc electrophoresis in 0.5 \times 10 cm columns containing 5 cm of a 10 per cent separatory gel (pH 9) overlaid with 3 cm of 3.5 per cent stacking gel (pH 6.5). The dialysed and freeze-dried active component was resuspended in 0.1 M potassium citrate and separated by gel filtration on 'Sephadex G-100' columns (2 \times 60 cm) using 0.1 M potassium citrate (pH 7.0) as the eluent. The antigen was again located, titred and checked for heterogeneity. Preparative acrylamide disc electrophoretic separation of the active component from 'Sephadex G-100' columns was carried out at 14° C with PD2/150 columns (Canalco, Corp.) with a 6 cm, 10 per cent, separatory gel (pH 9) overlaid with a 3 cm, 3.5 per cent, stacking gel (pH 6.5), using a constant current of 20 mA. The antigen was located by immunodiffusion and its heterogeneity determined by analytical acrylamide disc and immunoelectrophoresis. The purified antigen was subjected to electrophoresis on agar gel, fixed and stained for carbohydrate, protein and lipid by the periodic acid α -naphthol-p-phenylenediamine reaction, amido black and sudan black, respectively⁸. Appropriate standards were tested simultaneously. Different aliquots of skimmed milk (40 μ l. of 110 mg protein per ml.) were treated with 2 μ l. of pronase (100 mg/ml.) or amylase (375 mg/ml.) in 0.15 M NaCl–0.05 M Tris–0.06 M CaCl₂ buffer (pH 7.1), with neuraminidase (103 units/ml.) in 0.1 M acetate buffer (pH 5.0) and lysozyme (56.2 mg/ml.) in 0.1 M phosphate buffer (pH 7.4) and incubated for 1 h at 37° C. The antigen was hydrolysed with 2 M HCl for 8 h at 100° C in a sealed tube and the reducing monosaccharides were determined by the arsenomolybdate reaction⁹.

The degree of purification of the antigen at each step of the experimental procedure is shown in Table 1. The isolate from preparative disc electrophoresis represents an approximate purification of 260 fold. Analytical acrylamide disc electrophoresis of the isolate resulted in a single line with a possible resolvable contaminant of less than 1 per cent. Immunoelectrophoresis of this preparation resulted in a single immunoprecipitin line in the β_2 globulin region when reacted with unabsorbed rabbit antiserum (Fig. 1). Electrophoretic migration of the antigen on agar gel gives a single component staining for protein and carbohydrate. The weight ratio of protein to reducing monosaccharide was 5 : 3. The antigen did not

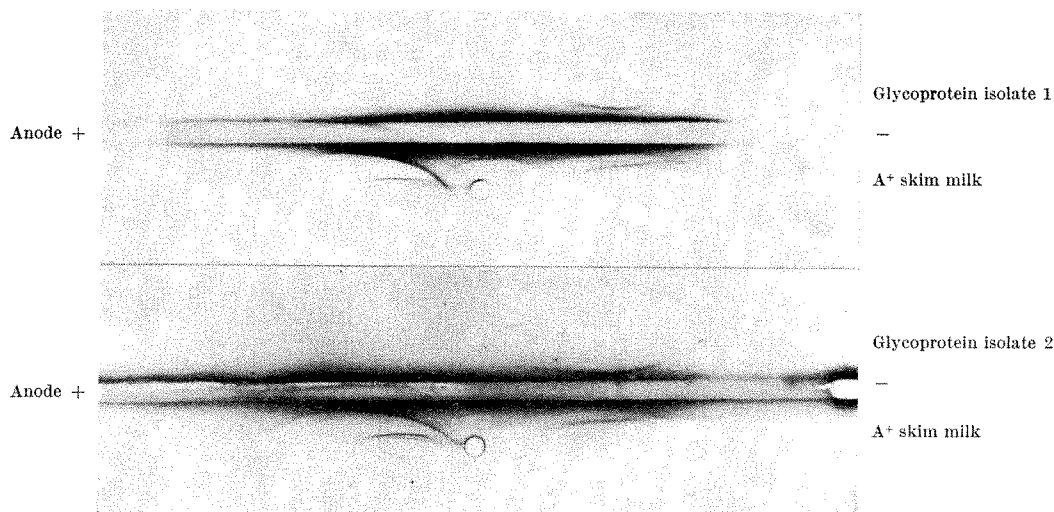


Fig. 1. Immunoelectrophoresis of glycoprotein from two isolation experiments and skim milk from A⁺ mice diffused against unabsorbed rabbit antiserum to A⁺ skim milk. The anode was on the left.

Experimental conditions	Antigenicity* (μ g protein/ μ l.)	Increase in antigenicity
Skimmed milk	45	1.0
CaCl ₂ precipitation		
Supernatant (whey)	12	3.2
Pellet	26	1.7
NH ₄ SO ₄ Precipitation		
0-30 per cent pellet	50	1.0
30-60 per cent pellet	12	3.2
60 per cent supernatant	(negative)	—
ECTEOLA-cellulose chromatography		
Fraction 24-56 ml.	6	7.5
'Sephadex G-100'		
Fraction 144-200 ml.	5	9.0
Preparative disc electrophoresis		
Fraction 60-84	0.17	260.0

The figures represent the pooled results of three isolations.

* Antigenicity = the lowest concentration of protein producing a positive immunoprecipitin line with standardized, specific rabbit antiserum.

stain for lipid. Strain A⁻ milk processed in an identical manner as the A⁺ yielded a glycoprotein which is chemically and physically identical. The glycoproteins from A⁺ and A⁻ milk are only distinguishable by immunological procedures. The antigen was not inactivated by neuraminidase, amylase, lysozyme and chloroform : methanol (2 : 1) extraction but was inactivated when treated for 1 h with either 70° C or pronase at 37° C. This glycoprotein represents the only unique immunochemical determinant that we have been able to find in milk containing the mammary tumour agent (Fig. 2).

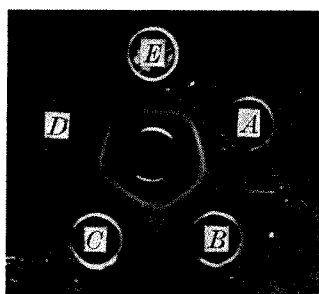


Fig. 2. Immunological identity of isolated glycoprotein; antigen from B particles ($\rho=1.12$), upper part of 'Ficoll' gradient ($\rho=1.07$), supernatant of clarified skim milk after ultracentrifugation at 100,000g for 1 h and fat globule membranes. B particles were prepared from 'Ficoll' gradients as previously described⁴. Cream which had been washed three times with 0.2 M sucrose containing 2×10^{-3} M MgCl₂, chilled and churned for 6 min at 7° C was separated into butter and buttermilk by centrifugation at 800g for 10 min at 5° C. The fat globule membranes were sedimented from buttermilk by centrifugation at 100,000g for 90 min. A, isolated glycoprotein; B, B particles from 'Ficoll' gradient; C, upper part of 'Ficoll' gradient ($\rho=1.07$); D, supernatant of clarified skim milk after ultracentrifugation; E, fat globule membranes. Centre well: specific A⁺ milk antiserum.

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Differential Toxicity on Normal and Transformed Cells *in vitro* and Inhibition of Tumour Development *in vivo* by Concanavalin A

THE carbohydrate binding protein concanavalin A (Con A)¹⁻³ can agglutinate cells transformed by a variety of carcinogenic agents, but it only agglutinates normal cells after they have been treated with trypsin⁴. This agglutination was reversed by competition with α -methyl-D-glucopyranoside (α -MG). These results indicated that transformed cells contain exposed binding sites that interact with Con A, whereas most such sites on normal cells are in a cryptic form^{4,5}. These experiments were undertaken to determine whether treatment with Con A can result in cell toxicity; and if so, whether the difference in structure of the surface membrane between normal and transformed cells can result in a differential toxic effect, and can be used to inhibit tumour development *in vivo*.

We used normal cells from secondary cultures of golden hamster embryos; lines of golden hamster embryo cells transformed *in vitro* by polyoma virus or after treatment with the chemical carcinogen dimethylnitrosamine (DMNA)²; a line derived from a simian virus 40 (SV40) induced hamster tumour; untransformed 3T3⁷ cells; and lines of 3T3 cells transformed by polyoma virus, SV40, or both polyoma and SV40. Cells were cultured in plastic Petri dishes in Eagle's medium with a four-fold concentration of amino-acids and vitamins and 10 per cent calf serum. Cells were seeded for experiments 3–4 days after subculturing at a seeding level of $2 \times 10^5 - 4 \times 10^5$ cells per 50 mm Petri dish. Dissociation of cells for routine subcultures were made with a 0.25 per cent (1:300) trypsin solution; cells were dissociated for experiments with a 0.02 per cent disodium versenate solution⁴. Con A was prepared (by Miles-Yeda) from Jack bean meal by two crystallizations as described¹, lyophilized and stored at -20°C . For each *in vitro* experiment the protein was freshly dissolved in phosphate-buffered saline (PBS) at 5–6 mg/ml., centrifuged at 500 g for 5 min, and the final concentration of Con A in the supernatant was determined by its absorbance at 280 nm (1 mg corresponds to 1.3 A unit). For the *in vivo* experiments, with higher concentrations of Con A, the material was used as a suspension.

To test the toxic effect of Con A on cells *in vitro*, the protein was added in 1.5 ml. of medium without serum. Pretreatment of Con A with α -MG was found to inhibit toxicity, and in order to control the action of Con A at various times after treatment, 4–5 ml. medium with 10 per cent foetal calf serum and α -MG at a concentration of 0.1 M was added to each plate. The number of cells was counted 24 h after the addition of Con A. Cells in Con A treated cultures were more readily removed from the plates for counting when they were incubated with 0.5 ml. of 1 M α -MG for 5 min before trypsinization, and this was used in all experiments.

Results obtained with the addition of α -MG at 2, 4 and 8 h after treatment with up to 1,000 μg Con A/ml. have indicated that at 50 μg /ml. for the hamster series and 1,000 μg /ml. for the 3T3 series there was a differential toxic effect on the transformed and untransformed cells (Fig. 1). This differential toxicity was clearly seen with the hamster cells after 8 h with 50 μg /ml. At 400 μg /ml. there was a greater toxicity on the transformed cells after 2 h, but the same toxic effect on normal and transformed cells after 4 or 8 h treatment. The 3T3 untransformed cells were more resistant to Con A treatment than normal hamster cells and exhibited no effects of toxicity even at 1,000 μg /ml. In nine experiments, with 8 h treatment at 500 μg /ml., the number of cells as a percentage of the control ranged from 80–100 per cent for untransformed 3T3 cells and from 3–33 per cent for SV40 transformed 3T3 cells. Cultures from one such experiment are shown in Fig. 2.

The viability of the cells surviving 8 h of Con A treatment at the concentrations shown in Fig. 1 was measured by their colony forming ability at the time when the number of cells per culture was counted. The cloning efficiency of Con A treated cells on X-irradiated (4,000 r) rat embryo feeder layers, as a percentage of untreated cells, was 84 per cent for normal hamster, 51–76 per cent for the different lines of transformed hamster, 94 per cent for untransformed 3T3 and 47–82 per cent for the different lines of transformed 3T3

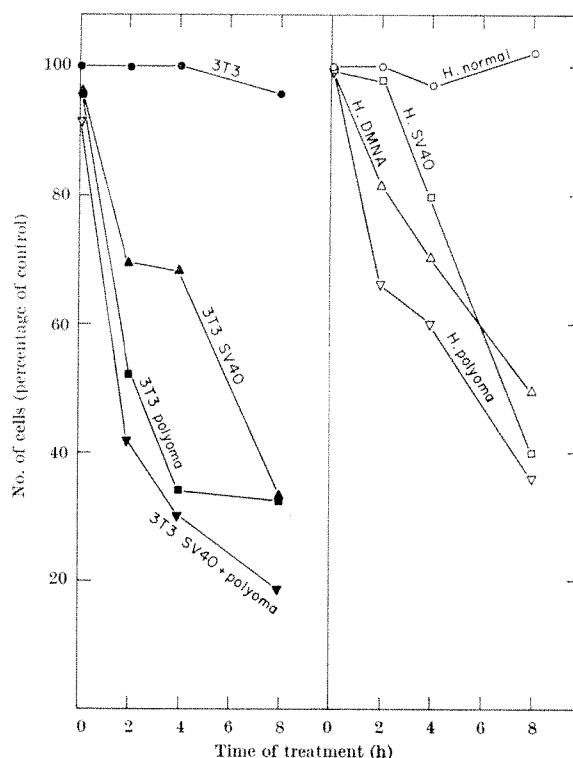


Fig. 1. Differential toxic effect of Con A on cells *in vitro* after different times of treatment. Hamster (H) normal and transformed cells were treated with 50 μg /ml., and 3T3 untransformed and transformed cells with 1,000 μg /ml. Con A. A solution containing 0.1 M α -MG was added at 0, 2, 4 and 8 h, and this was taken as the time of treatment with Con A. The number of cells were counted 24 h after the addition of Con A. Cells which had detached from the Petri dishes were also counted. There was a significant number of such cells only in the treated 3T3 SV40-polyoma cultures, and in this case these floating cells were also included in the results.

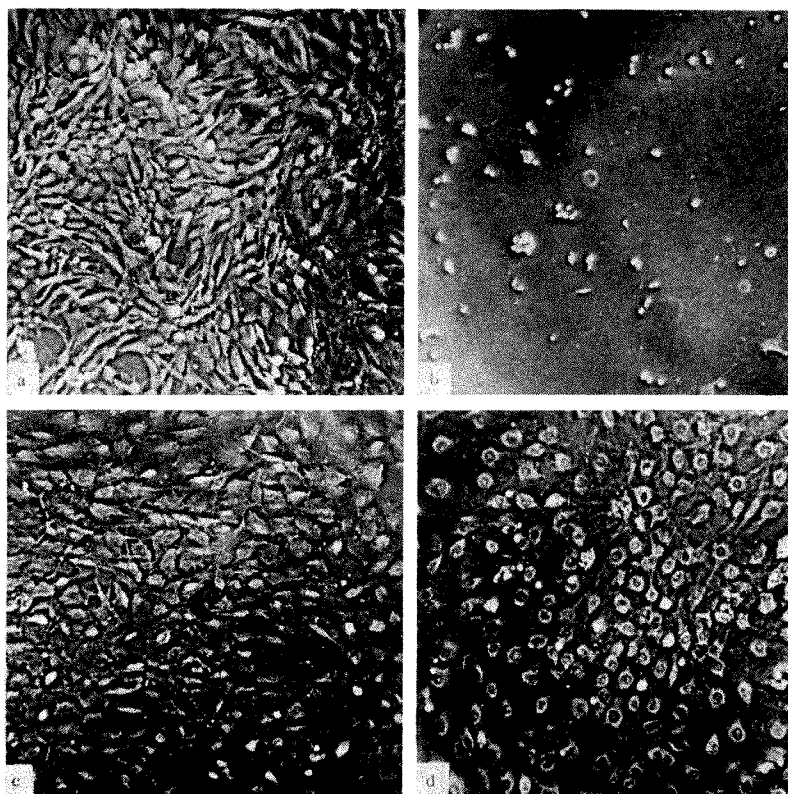


Fig. 2. Cultures of cells 48 h after treatment with Con A (500 μg /ml. with the addition of 0.1 M α -MG after 8 h). a and b, 3T3-SV40 cells, a without, and b with Con A treatment; c and d, 3T3 untransformed cells, c without, and d with Con A treatment ($\times 72$).

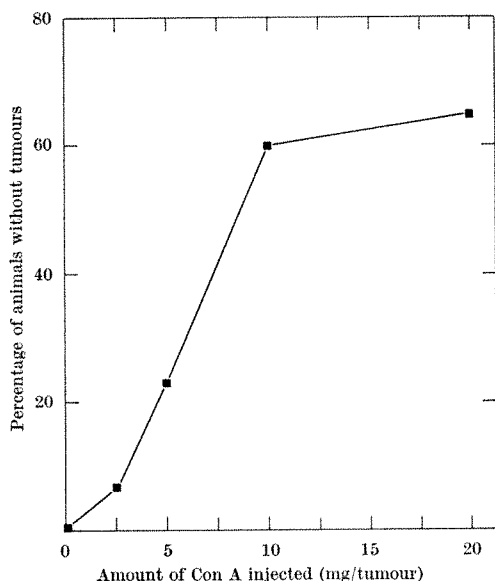


Fig. 3. Inhibition of tumour development by local injection of Con A into tumours produced after subcutaneous inoculation of 1×10^6 polyoma transformed cells into 5-6 week old hamsters. Con A was injected at 10 days after cell inoculation, and the results are from animals scored at 150 days, that is, at 104 days after the last control animal with tumours had died. Each point is the average of 25-30 animals from two experiments.

cells. Thus the principal toxic effect of Con A was on the total number of cells in the cultures, either by inhibiting cell multiplication or by cell killing.

Inhibition of tumour development by Con A *in vivo* was tested with tumours produced after subcutaneous inoculation of 1×10^6 polyoma transformed cells into 5-6 week-old hamsters. Con A was injected into the tumours in 0.5 ml. PBS at 10 days after cell inoculation, when the tumours were 3-6 mm in diameter. Subcutaneous injections of 1 mg Con A into hamsters without tumours produced a local reaction on the normal tissue. After 1 day there was an acute inflammatory process in the subcutaneous tissue with leucocyte infiltration, oedema and necrosis, that is, all the elements of an abscess, palpable as a soft swelling. The abscess continued to grow; it usually burst on the seventh day and an ulcer developed. There was complete repair of the tissue at about 14 days. It has also been reported that an Arthus-like reaction was obtained after injection of Con A into the skin⁸.

Injection of 10 or 20 mg Con A produced a local reaction that was not more extensive than 1 mg. At these doses, 70 per cent of sixty animals had no palpable tumours at 33 days after cell inoculation and the average size of the remaining treated tumours was about 4-25 per cent of the controls. Sixty-two per cent of the animals were still without tumours at 150 days (Fig. 3), that is, 104 days after the last control animal with tumours had died. Injection of 10 mg Con A in a solution of 2 M α -MG did not prevent a local reaction or the destruction of tumours, and there was also no inhibition of these effects by injection of demetallized Con A⁴. The concanavalin-glycoside complex may, however, have dissociated and the demetallized protein may have acquired new metal ions *in vivo*. Subcutaneous injection of Con A at a distance of about 2 cm from the tumour did not inhibit tumour growth even with 40 mg. Experiments with the hamster DMNA and SV40 cell lines indicated that inhibition of tumours produced by these cells required a local injection of 40 mg Con A. At this dose, 80 per cent of twenty animals had no palpable tumours at 33 days after cell inoculation.

The results indicate that the difference in the structure of the surface membrane between normal and transformed cells, as far as the α -MG-like binding sites of Con A are concerned, can be reflected by a differential toxic

effect of Con A on normal and transformed cells *in vitro*. This suggests that chemicals that interact differentially with transformed and normal cells may be useful in tumour therapy. It would be of interest to extend the present results on the inhibition of tumour development by Con A to chemicals such as wheatgerm⁹ and soybean¹⁰ agglutinins that interact with different carbohydrate-containing sites, and to chemicals that interact with other sites on the cell surface. It will also be of interest to determine to what extent the inhibition of tumour development by Con A *in vivo* is attributable only to a direct toxic effect on the cells, or also involves an immune reaction against cells coated with the protein.

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Failure of Certain Cytotoxic Lymphocytes to respond mitotically to Phytohaemagglutinin

CYTOTOXIC activity by lymphocytes has attracted considerable interest because of its possible role in the rejection of grafted tissues and neoplastic cells. Damage to target cell caused by lymphoid cells was first demonstrated in 1960 by Govaerts¹, and there were several subsequent reports of immunologically specific cytotoxicity towards target cells by lymphoid cells from sensitized donors. These have recently been reviewed extensively². The situation was complicated when cytotoxic activity by lymphocytes from apparently unsensitized donors was demonstrated in 1964 by Holm *et al.*³, in cultures of lymphoid cells and target cells when phytohaemagglutinin (PHA) was incorporated into the medium. Holm and Perlmann⁵ later showed that several other circumstances could lead to immunologically non-specific cytotoxic activity by lymphocytes. The induction of cytotoxicity in all these systems seemed to correlate well with increased mitogenic activity in the lymphocyte population tested. Recently it has been shown that immunologically non-specific cytotoxic activity can be evoked from lymphocytes when the target cell antigens are complexed with certain antibody⁵⁻⁷. This effect has been demonstrated in a wide variety of species including man⁸, and does not seem to require the participation of complement components. We have previously reported that much of the immunologically specific target cell damage by lymphocytes in rats is dependent on immunoglobulin sensitization

of target cells⁹. The sensitizing antibody, in this case, is synthesized by lymphocytes which are not themselves cytotoxic. Bubeník, Perlmann and Hašek¹⁰ have provided evidence that this mechanism brings about homograft rejection in donors made tolerant to graft antigens. Although there has been no report of antibody-induced cytotoxicity being associated with blast transformation, transformation in response to antigen-antibody complexes has been described^{11,12}.

In spite of the correlation between increased DNA synthesis and immunologically non-specific cytotoxicity which we have mentioned, there is strong evidence that these effects are not interdependent. Actinomycin D (ref 13), actinomycin C (ref 14) and X-irradiation (ref 15) in doses which effectively abolish DNA synthesis do not inhibit the PHA-induced cytotoxic activity of lymphocytes. Furthermore, the non-specific mitogen concanavalin A fails to induce cytotoxic activity in lymphocytes and actively suppresses that produced by PHA²².

We wish to present evidence that lymphocytes from unsensitized rats which efficiently damage antibody-treated target cells fail to increase DNA synthesis in response to PHA. This evidence is important for two reasons. First, it has been assumed on several occasions that lymphoid cells which have entered cell division in response to a stimulus would automatically have cytotoxic activity as a result of the same stimulus. Second, the inference has been made that because the lymphocyte which enters cell division in response to PHA is dependent on the presence of the thymus, at least in the mouse, cells which respond to PHA with non-specific cytotoxic activity are also thymus-dependent.

The cytotoxic assay system used in this investigation involved the measurement of chromium-51 released from labelled target cells. The culture period was 18 h and each culture contained 10^4 target cells. The cytotoxic effect of varying numbers of lymphoid cells, between 10^5 and 10^7 , was measured. The lymphoid cells were derived from an unsensitized inbred strain of black-hooded rats, and target cells were a continuous cell line of human origin, Chang cells. A pool of anti-Chang antiserum was obtained from rats immunized 14 days previously with 10^8 Chang cells given by intraperitoneal injection. Full details of these methods have been given elsewhere⁷. Thoracic ducts were cannulated by the method of Bolman *et al.*¹⁶, and lymphocytes were collected during 4 to 6 h periods into 20 ml. of Parker 199 medium containing 20 U of heparin/ml. The cytotoxic effect of thoracic duct lymphocytes was compared with that of lymphocytes from other sites which were prepared when the collection of lymph from the thoracic duct began, and they were stored in an equal volume of collecting fluid for the same time. In cultures measuring the incorporation of tritiated thymidine into lymphoid cells $1 \mu\text{Ci/ml.}$ of tritiated thymidine (specific activity 80 Ci/mmol) was added to each culture 4 h before the cultures were terminated after 72 h. The incorporated isotope was extracted by freezing and thawing the cells in isotonic saline, precipitation and re-washing in 5 per cent trichloroacetic acid and dehydration in ethanol. The resulting precipitate was dissolved in 0.3 ml. of 0.1 M NaOH and 0.2 ml. of this solution was counted in 10 ml. of scintillation fluid¹⁷. The results were subtracted from the counts obtained in cultures to which isotope had been added after 72 h.

By comparing the mitogenic response to PHA with antibody-induced cytotoxic activity in lymphoid cells from various sites, we found that some lymphocytes which responded to PHA did not damage antibody complexed target cells (Table 1). Cells from the thymus and thoracic duct lymph responded well to PHA, but failed to damage Chang cells in the presence of anti-Chang antibody. The data in Table 1 do not exclude the possibility that cytotoxic lymphocytes will respond to PHA by beginning increased DNA synthesis, but the following

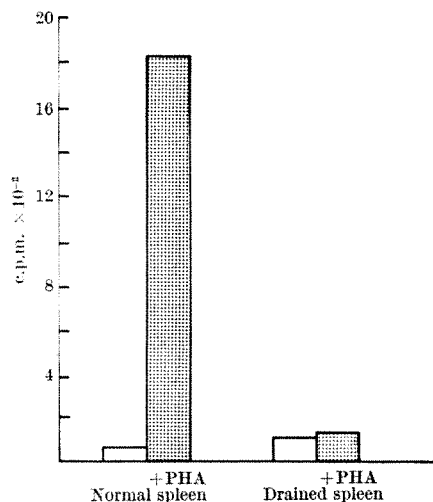


Fig. 1. Mitogenic effect of PHA on normal spleen cells and on spleen cells from rats depleted by drainage of thoracic duct. For explanation see text.

experiments suggest that this is not the case. Iversen¹⁸ reported that blood lymphocytes from rats which had been depleted of lymphocytes by thoracic duct drainage for 3 days had no mitogenic response to PHA. Fig. 1 is representative of nine experiments comparing the incorporation of tritiated thymidine, in culture with and without PHA, of (a) normal spleen cells and (b) spleen cells from rats depleted of lymphocytes by thoracic duct drainage for 4 days. The former population consistently responded well to PHA, but depleted spleen cells at best only produced a marginal increase in DNA synthesis. Fig. 2 shows that depleted spleens, far from losing their ability to damage antibody complexed target cells, had increased cytotoxic capacity. In eight experiments of the type represented in Fig. 2a spleen cells from depleted animals always had greater cytotoxic capacity for a given number of mononuclear cells than did those from normal rats.

To exclude the possibility that this cytotoxic effect was caused by macrophages, peritoneal exudate cells from normal rats were investigated. These cells comprise 85 per cent or more macrophages. By sequential absorption onto glass beads, preparations were obtained containing less than 0.3 per cent cells that would adhere to coverslips in 12 h cultures. The cytotoxic capacity of the macrophage-depleted exudate cells was at least as good as that of unabsorbed preparations. These results suggest that the principal cytotoxic effector cell is lymphocytic although it cannot be excluded that certain macrophages may also be able to damage antibody complexed target cells. This reservation must be made because the macrophage-depleted exudate cell population did not show as great an increase in cytotoxic capacity as would have been expected if no cytotoxic cells had been lost by absorption onto glass beads.

Table 1. MITOGENIC RESPONSE TO PHA AND CYTOTOXIC ACTIVITY AGAINST ANTIBODY COATED TARGET CELLS IN VARIOUS LYMPHOID POPULATIONS

	Mitotic response to PHA	Cytotoxic effect towards Chang cells
Thymus	++++	—
Thoracic duct	+++	—
Lymph node	+++	+
Bone marrow	—	+
Blood	+++	++
Peritoneal exudate	+++	+++
Spleen	+++	+++

Mitogenic response was measured by incorporation of tritiated thymidine in 72 h cultures each with 10^7 lymphoid cells. Cytotoxicity, as assessed by release of ⁵¹Cr, was compared using different ratios of lymphoid cells to target cells, as in Fig. 2. Normal spleen cells were included in each experiment as a positive reference population. Thoracic duct and thymus cells consistently slightly decreased the release of ⁵¹Cr from target cells. Exact comparative data are not presented, for it is not possible to obtain all these lymphoid tissues from a single rat.

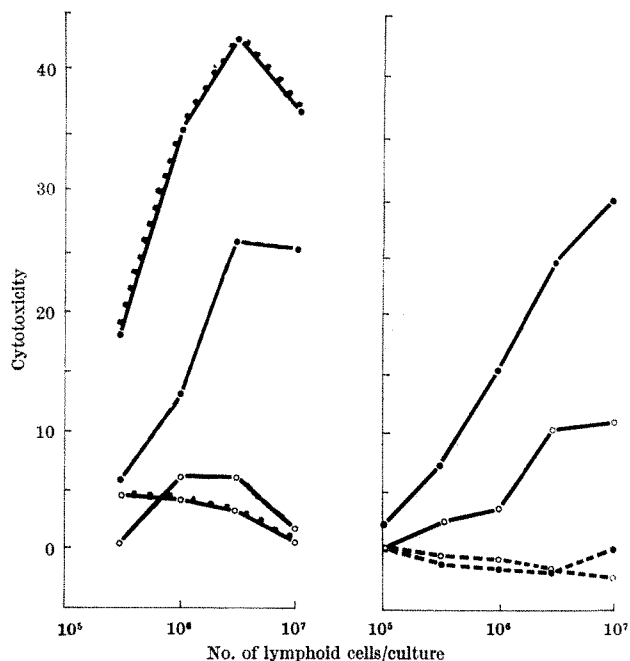


Fig. 2. Ability of different populations of lymphoid cells from unimmunized rats to destroy antibody coated Chang cells. The cultures measured the release of ^{51}Cr from 10^4 target cells. Cytotoxicity is the difference between the percentage release from target cells only and from cultures with lymphoid cells. ●, Cultures with 1 : 1,000 anti-Chang antibody; ○, cultures without anti-Chang antibody; ---, spleen cells from rats depleted by thoracic duct drainage for 4 days; —, normal spleen cells; ---, thoracic duct lymphocytes.

Our results strongly suggest that lymphocytes damage antibody complexed target cells and that these effector cells do not have a mitogenic response to PHA. We have shown that soluble cytostatic factors, such as those described by Ruddie and Waksman¹⁹, are unlikely to be responsible for the cytotoxic effect that we have described here⁹. It is probably significant that Ruddie and Waksman used lymph node lymphocytes. In our system these are a poor source of cytotoxic cells. In addition Granger²⁰ has shown that production of a similar factor by both murine and human lymphoid cells is inhibited by puromycin. Holm¹³ and ourselves⁹ have shown that cytotoxic activity induced by PHA or antibody is not prevented by doses of puromycin which are efficient at preventing antibody synthesis. It therefore seems reasonable to conclude that the production of soluble factors is an independent cytotoxic mechanism from that which we have described.

Cerottini, Nordin and Brunner²¹ have investigated the origin of cells in the mouse which show immunologically specific cytotoxic activity towards allogeneic tumour cells. They provide evidence that the cytotoxic cell is derived from the thymus and requires the cooperation of no other cell type. They have not been able to attribute this cytotoxic effect to soluble mediators. If population studies in the mouse can be applied to the rat then the population data we present does not favour the conclusion that the cytotoxic effector cell in our system is of thymus origin. If this inference is correct, then it seems that there may be at least three cytotoxic mechanisms which involve lymphocytes.

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Release of Elastolytic Activity from Blood Platelets

THE degradation of elastin early in atherosclerosis¹ has been attributed to elastolytic enzymes of the blood or vessel wall², but no enzyme other than pancreatic elastase (EC 3.4.4.7) (ref. 2) has yet been demonstrated conclusively. We have recently reported the presence of an elastolytic enzyme in blood platelets³ and now present evidence that it can be released from blood platelets by certain factors which produce aggregation and viscous metamorphosis of platelets. The elastolytic enzyme(s) can be separated from acid cathepsins and partially purified⁴.

Most of the methods used have been described before³⁻⁶. Elastolytic activity was determined using elastin labelled with iodine-125 (ref. 6). Catheptic activity was determined by a modification of Anson's method using haemoglobin as substrate at pH 3.5 (ref. 5).

Human blood platelets, washed in saline and Tris buffer, were resuspended in 0.1 M Tris-HCl buffer (pH 7.5) and uniformly distributed in a final volume of 5 ml. The Tris-HCl buffer contained either ADP, adrenaline, collagen or 'Triton X-100' and was incubated for 15 min at 30° C while being shaken. The platelet suspensions were then centrifuged at 3,000 r.p.m. in the cold, the particulate fraction was washed twice with 0.1 M Tris-HCl buffer (pH 8.6) and the elastolytic activity of the washed and resuspended particulate fraction and of the supernatant (adjusted to pH 8.6 with 1 M Tris) was determined^{3,4,6}. Appropriate control incubations were included in every experiment (elastin plus buffer only, and platelets that had not been preincubated). A sonicated platelet suspension (30s at 0° C, 1.5 A) was kept at 4° C for 15 min, centrifuged and assayed as described.

Considerable enzyme activity was released during incubation in the presence of ADP or 'Triton X-100' or by sonication. Platelets incubated with buffer alone gave the base line value for the calculation of the percentage increase of enzyme activity determined in the supernatant and particulate fractions in the presence of the different releasing agents (Table 2). 'Triton X-100' and sonication produced the greatest increase of total activity. The distribution between the particulate and supernatant fractions was equal with either collagen or ADP; with adrenaline, 'Triton' or with sonication most of the activity was in the supernatant fraction.

The data in Tables 1 and 2 suggest two phenomena: release of enzyme activity from platelets, and increase of total activity in the particulate and supernatant fractions. The release of the enzyme can be explained by assuming

Table 1. SPECIFIC ELASTOLYTIC ACTIVITY OF PLATELET EXTRACTS

Platelet preparations	Specific elastolytic activity (c.p.m./mg of protein)
Platelet suspension, not incubated	1,380
Tris-HCl (pH 7.5) extract of a platelet suspension incubated at 30° C for 15 min	13,150
Tris-HCl (pH 7.5) extract of a platelet suspension incubated at 30° C for 15 min in the presence of 1.0 mg of ADP	19,400
Tris-HCl (pH 7.5) extract of a platelet suspension incubated at 30° C for 15 min in the presence of 40 mg of 'Triton'	28,850
Tris-HCl (pH 7.5) extract of a sonicated platelet suspension	24,130
Platelet extracts were incubated with ¹²⁵ I-elastin for 2 h at 37° C in Tris-HCl buffer (pH 8.6)*.	

Table 2. RELEASE OF ELASTOLYTIC ACTIVITY FROM BLOOD PLATELETS

Releasing agent	mg added	Increase of total activity (per cent)	Total activity in particulate supernatant (per cent)
Collagen	0.2	214	54
ADP	1.0	35	50
Adrenaline	0.050	32	18
'Triton X-100' (a)	20	174	7
(b)	40	416	16
Sonication		425	3

The incubation mixture consisted of 1 ml. of platelet suspension containing 10.7 mg of protein, 1 ml. of the activating agent and 2 ml. of Tris-HCl buffer, pH 7.5. Incubation was for 15 min, with shaking at 30° C. The suspension was then centrifuged, the particulate fraction washed twice with Tris-HCl buffer, pH 8.6 and taken up in the same buffer. The supernatant was adjusted to pH 8.6 and both supernatant and particulate fractions were tested for elastolytic activity as described.

that the elastolytic enzyme is associated with a particulate subcellular fraction, not accessible to the fibrous elastin substrate. Release of elastolytic activity could be analogous to the liberation of lysosomal enzymes⁷. Increase of total enzyme activity could be due to the transformation of an inactive precursor form of the enzyme by the activation process into an active form.

Elastolytic and catheptic activity were separated by ammonium sulphate fractionation of the 'Triton X-100' extract (Table 3).

Table 3. DISTRIBUTION OF ELASTOLYTIC AND CATHEPTIC ACTIVITY IN (NH₄)₂SO₄ FRACTIONS OF BLOOD PLATELET EXTRACTS

(NH ₄) ₂ SO ₄	Elastolytic activity (c.p.m./mg of protein)	Total in fraction (per cent)	Catheptic activity* (mg Hb/mg of protein)	Total in fraction (per cent)
'Triton X-100' extract	21,400	100	3.0	100
40 per cent (NH ₄) ₂ SO ₄ precipitate	3,500	15	2.67	63
60 per cent (NH ₄) ₂ SO ₄ precipitate	5,700	8.3	0.62	5.8
60 per cent (NH ₄) ₂ SO ₄ supernatant	22,400	52	0	0

* At pH 3.50

Elastolytic activity was present chiefly in the supernatant of the 60 per cent ammonium sulphate fraction and catheptic activity was concentrated in the 40 per cent ammonium sulphate precipitate.

The failure to obtain an increased specific activity by purification could be explained by the stabilization and/or activation of the enzymes by 'Triton X-100' later eliminated during the purification process. Both the 40 and the 60 per cent ammonium sulphate precipitates were subjected to gel filtration on columns of 'Sephadex G-100' and 'G-200'. Most catheptic activity was concentrated in the first peak, eluted with the exclusion volume from the column loaded with the 40 per cent ammonium sulphate precipitate. The elastolytic activity of the 60 per cent ammonium sulphate precipitate was recovered in two peaks, one within the void volume and a second eluted much later⁴.

The specific activity of the first peak was 20,000 c.p.m./mg of protein. The specific activity of the second was about 140,000 c.p.m./mg of protein. The height of the second peak was about 10 per cent of that of the first peak. The distribution of elastolytic activity over several protein fractions could mean that more than one enzyme was present, or that the same enzyme was present in several molecular forms, differing in size.

The highest specific elastolytic activity recovered from platelet extracts was associated with the protein fractions

present in the 60 per cent ammonium sulphate supernatant. The total amount of protein in this fraction was 4–5 mg, obtained from 200–300 mg of total platelet protein extract, representing 10 to 12 l. of blood from the same donor (obtained by plasmapheresis).

Several substances were assayed in order to activate or inhibit the purified elastolytic factor of blood platelets. The elastolytic activity of a purified fraction obtained by gel filtration on a column of 'Sephadex G-200' from the 60 per cent ammonium sulphate precipitate was strongly inhibited by low concentration of CaCl₂; 30 μ M gave about 70–80 per cent inhibition. In the presence of CaCl₂ this protein solution became turbid. There was only slight activation with 30 μ M EDTA, ADP and 'Triton X-100' (~30 per cent). Adrenaline and 5-hydroxytryptamine had no effect.

Adrenaline and ADP, both active in platelet aggregation, clearly have different actions on the elastolytic activity of intact platelets and on the purified enzyme. These results, together with earlier findings^{3,4}, suggest that human blood platelets contain an enzyme attacking elastin, which did not cause the complete "dissolution" of particulate elastin in 24–48 h. This enzyme is different from the cathepsin(s) having a pH optimum of 3.5.

The fact that the enzyme(s) can be activated and/or released by substances capable of producing platelet aggregation, such as collagen or ADP, suggests an involvement in the pathological degradation of elastin of the arterial wall. According to the thrombogenic theory⁸, a thrombus should be formed and muralized to produce the sclerotic lesion, but if this elastolytic factor is involved, it could do it without a permanent thrombus formation by releasing elastolytic enzyme(s) at or near the arterial wall. The liberated enzyme could then penetrate the arterial wall and cause local degradation.

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Solubilization and Partial Purification of Human Leukaemic Specific Antigens

Using the mixed lymphocyte reaction, we have reported evidence for the existence of neo-antigens on human leukaemic cells¹. These observations have been confirmed by others using similar techniques^{2,3}. There is now convincing evidence for the existence of tumour specific antigens in humans^{4–6} and in addition mouse leukaemic antigens have been solubilized and purified^{7,8}. We have attempted to extract and solubilize human leukaemia specific antigens, because solubilization is an essential prerequisite for purification.

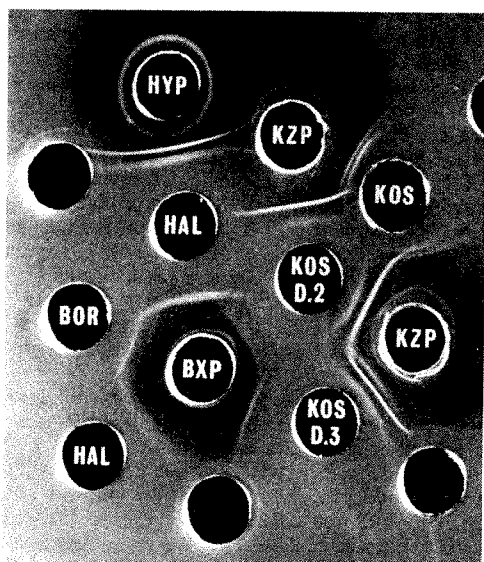


Fig. 1. Example of reactivity of rabbit heteroantisera, prepared by immunization with solubilized human leukaemic material, against various leukaemic preparations. All antisera have been absorbed with NHS, pool of HL-A and inactivated papain. All leukaemic antigens are purified through 'Sephadex G-75' and 'G-200' columns. The preparations KOS D.2 and KOS D.3 are the two active DEAE-cellulose fractions.

A soluble preparation could be assessed for leukaemic specificity in an autologous system by its ability to stimulate blast formation of the patient's lymphocytes taken during remission. It has already been shown that soluble HL-A antigens can stimulate allogeneic lymphocytes *in vitro*⁹. Neutralization of specific autologous leukaemic antiserum is a possibility arising from other approaches that have been made¹⁰. Both techniques are difficult, the former because of the rarity of patients in remission to provide cells on which to experiment, the latter because of the difficulty of obtaining suitable antisera.

Several reports have shown, nevertheless, that human leukaemic material can produce specific antibodies in a xenogeneic situation¹¹⁻¹⁴. Soluble preparations extracted from leukaemic blood could be used to immunize rabbits and the sera so obtained, after suitable absorption, could be tested by the immunodiffusion technique against various soluble preparations obtained from leukaemic individuals and non-leukaemics as controls.

Table 1. SOLUBLE LEUKAEMIC MATERIAL USED FOR THE PREPARATION OF RABBIT HETEROANTISERA

Code	Solubilized leukaemic preparation	White cell count/mm ³	Rabbit heteroantisera after absorption with NHS, HL-A and papain
BOR	Acute myeloblastic leukaemia	87,000	BXP
HAL	Sub-acute myeloblastic leukaemia	65% blasts 185,000	HYP
KOS	Chronic myeloid leukaemia	24.5% blasts 200,000	KZP
AVL	Chronic lymphatic leukaemia	150,000	AWP
FOS	Acute myeloblastic leukaemia	220,000 77% blasts	—

The rabbits were immunized by four subcutaneous injections at weekly intervals with 1.5 ml. of a solution of 1 mg/ml. of soluble antigen in saline with 2 ml. of Freund's complete adjuvant. All sera were absorbed with normal human serum (NHS) (60 mg/ml.), a pool of HL-A (30 mg/ml.) and inactivated papain (2 mg/ml.), because reactivity against these materials was observed.

White cells obtained from peripheral blood from five leukaemic patients with high blast cell counts were extracted separately (Table 1). The techniques of extraction and solubilization for H-2 (refs. 15, 16) and HL-A¹⁷⁻¹⁹ were used and have been described elsewhere. Briefly, cell membrane material from peripheral blood white cells was eluted and solubilized by the addition of papain. The soluble products thus obtained were purified through 'Sephadex G-75' and 'G-200' columns. Individual antisera against four of these preparations have so far been

prepared. Successive absorptions with normal human serum (NHS), soluble HL-A antigen pool and papain removed the reactivity of these antisera against a panel of soluble HL-A preparations obtained from spleens from twenty-one non-leukaemic individuals.

The antisera, after absorption, were tested against the five soluble leukaemic preparations and, although all gave positive reactions with the original material, each produced a different pattern of precipitation lines with the other preparations. The pattern of lines is receiving further study to elucidate reactions of identity and differences. Table 2 summarizes these results and indicates the number of lines obtained with each sera. Although the small amount of soluble material available prevented the extensive studies which are necessary to identify all the lines completely, the data show that some lines are common to leukaemias of different types.

Table 2. NUMBER OF LINES WITH EACH ANTISERA WHEN TESTED AGAINST THE FIVE LEUKAEMIC PREPARATIONS

Rabbit heteroantiserum	BOR	HAL	KOS	AVL	FOS
BXP	4	1	2	2	4
HYP	3	3	—	—	3
KZP	2	—	3	2	1
AWP	1	—	1	1	—

One serum (BXP) reacts with all five preparations. One serum (KZP) reacts with four of the preparations and both the other sera with three.

The presence of non-specific tumour-associated antigens²⁰ was excluded by the failure of the leukaemic antisera to react with materials known to contain these antigens, including ten sera from pregnant women, human ascitic fluid or malignant cells from pleural fluid. The results of the various tests carried out with the leukaemic antisera are summarized in Table 3.

Further purification of the leukaemic specificities from the preparation KOS was subsequently attempted. The soluble product was run through a DEAE-cellulose ion exchange column, eluted over a molarity gradient of Tris from 0.05 to 0.5 M (pH 8.0) and five fractions were recovered from successive molarity elution regions. These fractions were tested against the four leukaemic antisera by the immunodiffusion technique and activity was found only in the fractions eluted where transplantation antigens elute^{16,18}. The pattern of lines (Fig. 1) shows the DEAE-cellulose fractions and 'Sephadex G-200' fractions tested against the xenoantisera. It is perhaps significant that in the appropriate mouse leukaemic preparations the TL antigen appears in the same fractions, although it is premature to see this as evidence for homology between TL and human leukaemic antigen specificities.

The leukaemic antisera, after absorption with soluble HL-A material and NHS, were also tested for direct lymphocytotoxicity on a panel of ten healthy volunteers and gave negative results. Direct cytotoxicity on leukaemic cells has proved difficult to evaluate, for the cells available were poorly viable. Preliminary results suggest, however, that these sera do react with leukaemic cells but fail to react with normal cells. Our findings support the existence of leukaemic specific antigens on human leukaemic cells. These antigens can be extracted and solubilized and can be used to raise specific antibodies in a xenogeneic situation.

To determine whether this material can be used for diagnostic or therapeutic purposes, further investigation is needed. Purified solubilized leukaemic antigens might replace leukaemic cells in active immunotherapy^{1,21,22} and the xenoantisera might be used in passive immunotherapy. Leukaemic cells in long-term culture^{23,24} might serve as a source of leukaemic antigens in the same way as lymphocytes in culture are used as a source of HL-A antigens²⁵.

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Table 3. REACTIVITY OF RABBIT HETEROANTISERA PREPARED AGAINST SOLUBILIZED HUMAN LEUKAEMIC MATERIAL

Antisera (absorbed with NHS, HL-A and inactivated papain)	Soluble HL-A from non- leukaemic donor spleens (panel of 21)	Papain	NHS	BOR	Leukaemic antigens			FOS	Soluble material from non- leukaemic blood (panel of 3) volunteers)	Pregnant sera (panel of 10)	Human ascitic fluid	Human malignant cells from pleural fluid	Soluble antigen from huma thymuses (panel of 27)
					HAL	KOS	AVL						
BXP	—	—	—	+	+	—	+	+	—	—	—	—	—
HYP	—	—	—	+	+	—	+	+	—	—	—	—	—
KZP	—	—	—	+	—	—	+	+	—	—	—	—	—
AWP	—	—	—	+	—	—	—	—	—	—	—	—	—

—: no precipitation line
+: precipitation line occurs } in immunodiffusion

All antisera absorbed with NHS, pool of HL-A and inactivated papain before use. The absorbed sera were then tested against soluble material extracted from peripheral blood from three healthy volunteers, for all leukaemic extracts were obtained from peripheral blood while the HL-A pool used for absorption was from splenic origin. No reaction was observed. The possibility of a TL-like leukaemic specificity was investigated and 27 soluble extracts from human thymuses were tested but also with negative results.

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In vivo Specific Antigen Recognition by Rosette Forming Cells

THE rosette test has been used extensively for the study of antibody forming cells^{1,2}, but few data are available on rosette formation in unimmunized animals^{1,3}. Most "spontaneous" rosette forming cells (RFC) in the spleens of unimmunized mice are small lymphocytes⁴ bearing immunoglobulin receptors⁵ specific for the antigen³;

RFC against chicken erythrocytes are present before birth³. There is no correlation between the number of RFC in the spleen and the level of the corresponding humoral antibodies¹.

We have confirmed these data in C57 Bl6, CBA and Swiss mice and also demonstrated a remarkable sensitivity of these spontaneous RFC to antilymphocyte sera⁶ and certain immunosuppressive drugs⁷. The inhibition of spontaneous RFC by azathioprine was observed at 37° C (after *in vitro* activation of azathioprine with red blood cells), for non-cytotoxic concentrations as low as 10⁻⁹ M; moreover, it was reversible at 37° C, indicating that the receptors of the cell surface are produced by the RFC. Further, spontaneous RFC are inhibited in the presence of complement by anti-theta serum. A sample of this serum, kindly provided by M. Raff⁸, inhibited spontaneous RFC at the concentration of 1/500, whereas cytotoxicity was not observed on spleen cells even at the concentration of 1/10. This indicates the thymodependence of spontaneous RFC (ref. 8). Biozzi¹ and Laskov³ have suggested that spontaneous RFC could be natural antibody producing cells, but we suggest that spontaneous RFC, which are small lymphocytes producing cell bound antibody-like receptors, might be antigen sensitive cells as well⁹. It is assumed that some spontaneous rosette forming cells produce natural antibodies, as has been shown for plaque forming cells, but that some others are involved in the initial phase of the immune response, antigen recognition. To test this we have prepared, using a 'Ficoll'-'Triosil' gradient, spleen cell populations specifically depleted in RFC against one antigen and have tested their immunological competence in cyclophosphamide-treated mice.

The gradient used was that described by Harris¹⁰ for human lymphocytes—a mixture of 24 parts of 9 per cent 'Ficoll' (Pharmacia), and 10 parts of 34 per cent 'Triosil' 75 (sodium metrizoate, Glaxo). Spleen cells were prepared from unimmunized Swiss mice aged 5–9 weeks (H₂—Centre d'Élevage des Animaux de Laboratoire, Orléans, La Source). 100 × 10⁶ of these spleen cells were mixed with 400 × 10⁶ sheep red blood cells (SRBC) or 100 × 10⁶ chicken red blood cells (CRBC), and spun down for 5 min at 200g, to form the rosette, and finally resuspended by gentle agitation for 10 min⁶. RFC were counted in a small aliquot, and 1 ml. of the preparation was layered on the surface of 1 ml. 'Ficoll'-'Triosil' in a centrifuge tube. The tube was then spun down 20 min at 400g, after which a ring formed at the top of the gradient. This ring contained mostly spleen cells, with less than 10 per cent heterologous red blood cells. Conversely, the cell suspension present below the gradient contained mostly erythrocytes. Moreover, the ring was specifically depleted of RFC against the antigen used for rosette formation before passage on the gradient, whereas the suspension below the gradient was specifically enriched in these RFC. Table 1 gives the results of rosette counting before and after passage on the gradient, when rosettes against CRBC were formed before passage.

Swiss mice were injected intraperitoneally with 300 mg/kg of cyclophosphamide, as described by Santos¹¹.

Table 1. SEPARATION OF SPLEEN RFC BY A 'FICOLL-TRIOSIL' GRADIENT AFTER ROSETTE FORMATION WITH CRBC

	Number of RFC per 1,000 spleen cells	
	CRBC	SRBC
Original preparation	3.5	1.1
Enriched population	42	1.2
Depleted population	0.2	1.1

Six hours later, they were injected intravenously with a mixture of 20×10^6 spleen cells, 300×10^6 SRBC, and 100×10^6 CRBC. Spleen cells were given either unmodified, or after rosette formation against SRBC or CRBC, and subsequent treatment by the 'Ficoll'-'Triosil' gradient as described earlier. It was checked that the passage of spleen cells on the gradient without previous rosette formation, and rosette formation itself 30 min before injection to the mice, did not modify the immunocompetence of the cells. Seven days later, the immune response of the mice was tested by rosette formation in the spleen (performed as already described), and haemagglutinin titration in the serum (1 vol of inactivated serum dilution and 1 vol of 0.5 per cent SRBC or 0.5 per cent CRBC). The results are indicated in Figs. 1 and 2. It can be seen that the passage on the gradient induced a specific unresponsiveness to the type of red cells used for rosette formation, both at the humoral and cellular levels. Moreover, the depletion reached a level that is significantly lower than that observed in non-reconstituted mice ($P < 0.01$).

These experiments demonstrate that in unimmunized mice, a specific cell population exists, which is preconditioned to heterologous red blood cells, and this population is necessary for the immune response to these antigens, as suggested by Burnet¹². The presence of RFC against CRBC before birth, indicates that the preconditioning might be independent of any previous sensitization. Similar conclusions have already been put forward by Wigzell and Makela using specific depletion with antigen coated columns¹³, and by Ada and Byrt using I 125-labelled flagellin¹⁴, and it seems that the flagellin binding cells are very similar to our RFC (ref. 15). In Ada's experiment, however, it was not very clear whether the flagellin-binding cells were killed or simply inactivated (radiation induced tolerance). In our experiment, we have direct proof of the elimination of sheep RFC from the RFC content of the gradient-enriched spleen cell suspension.

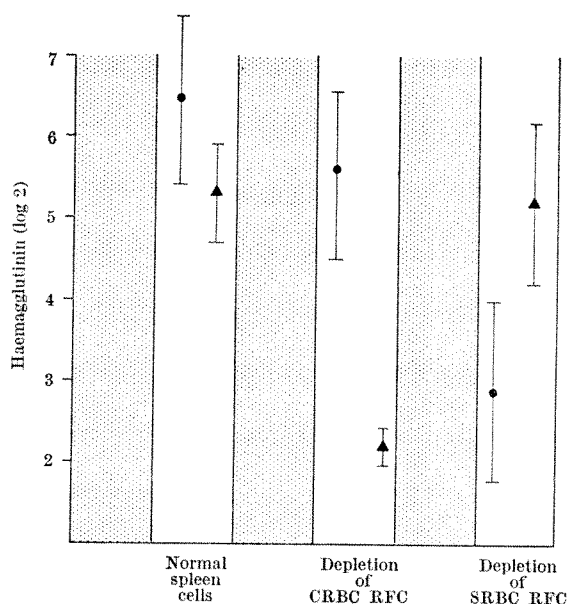


Fig. 1. Haemagglutinin response in cyclophosphamide treated mice restored by normal spleen cells or spleen cells depleted of their RFC against CRBC (▲) or SRBC (●). (Number of mice in each group: 25, 12, 10. Results are given ± 2 s.e.) $P < 0.01$.

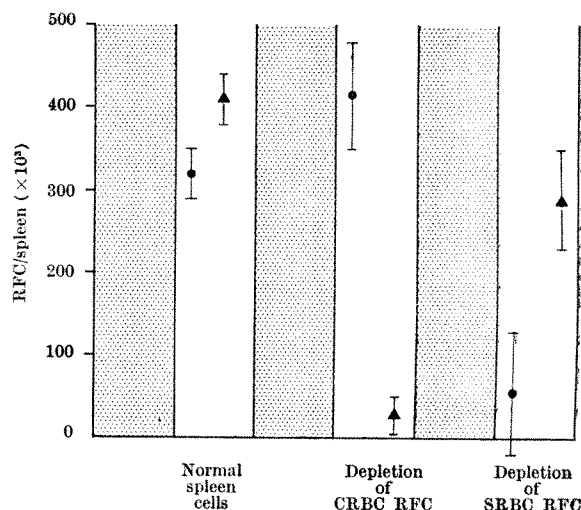


Fig. 2. Rosette forming cells response in cyclophosphamide treated mice restored by normal spleen cells or spleen cells depleted of their RFC against CRBC (▲) or SRBC (●). (Number of mice in each group: 24, 20, 10. Results are given ± 2 s.e.) $P < 0.01$.

The rosette forming cells seem thus to be involved either in antigen recognition or in antibody formation. In unimmunized animals, the majority of RFC would be antigen-sensitive cells, and a few natural antibody forming cells. In immunized animals, the antibody forming cells would be in higher proportion. It seems that antigen sensitive cells might be the target for antilymphocyte sera^{6,16}, purine analogues⁷, phytohaemagglutinin, polyribonuclease and asparaginase (our unpublished work), because of their high *in vitro* sensitivity to these agents in contrast with the relative *in vitro* resistance of plaque forming cells to the same agents. All these findings might be extended to the human situation in which, up to now, no major difference has been found with the mouse model^{17,18}, except the higher number of RFC found in man. This number is somewhat variable according to the different authors^{17,19} and it is possible that the significance of RFC differs with the technique. It could be that not all human RFC are specific antigen-sensitive cells, especially when high numbers are found.

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Variability, Structural Glycoproteins, and Classification of Herpes Simplex Viruses

HERPES simplex is an infectious disease of man caused by a virus once known by the same name but which is now euphemistically known as herpesvirus hominis¹. A characteristic of this disease is localization to particular parts of the body, frequently on the skin and near the lip, cornea, thigh or genitals. Immunological analysis² of herpes viruses isolated from a large number of patients has led to the suggestion that there are two types of herpes viruses. Viruses isolated from infections on all parts of the body except the genitals frequently fall into type 1. Conversely, viruses transmitted venereally and isolated from lesions on the genitals fall into type 2. Recently there has been considerable interest in the properties, classification and evolution of herpes viruses, particularly in view of the reports linking carcinoma of the cervix with type 2 herpes viruses^{3,4} and cancer of the lip with type 1 viruses^{5,6}. With respect to the extent of the relatedness of type 1 and type 2 viruses and the role of the virus in determining the site of infection, we suggest that (1) herpes simplex viruses in nature and in the laboratory form a spectrum of variants differing with respect to structural components of the virus and, in certain cases, biological properties and mode of transmission; (2) the viruses isolated from genital infections (type 2) are most probably closely related to viruses causing infection of the face (type 1); and (3) herpesvirus hominis is not a suitable name for herpes simplex virus.

Herpes simplex strains freshly isolated from patients and strains maintained in the laboratory for a long time differ with respect to their effects on cells⁷⁻⁹, morphologic aspects of development¹⁰, buoyant density in CsCl, heat stability and other biological characteristics^{7,11,12}. The classification of herpes viruses into two types is based on serological tests^{2,13,14} but previous evidence has shown that immunological specificity in neutralization tests, effects on the behaviour of infected cells, and certain physical characteristics of the virus are covariant properties dependent on the structure of the surface of the virus^{7,15-17}. We consider that herpes simplex viruses are not bisected into two types, but rather, that they form a continuously variable spectrum and reflect an intrinsic variability of the envelope glycoproteins of the virus. The evidence in support of this conclusion is as follows:

(1) The term "type" obscures the fact that herpes viruses segregated into the two groups can be neutralized by heterologous antisera^{2,7,8,14}. The differentiation between type 1 and type 2 is not nearly as absolute as between poliovirus types 1, 2 and 3, or between influenza A and B. Rather, the differences are more nearly those that have been observed among different type 1 poliovirus strains¹⁸ or among different variants of influenza virus within a single epidemic. Two observations support this: first, it has been reported that a type 2 variant of strain HF gave rise to a Type 1 (HPF) strain². Second, virus strains intermediate between the so-called type 1 and type 2 strains have been described. An example of an intermediate strain is mutant MP derived from mP, a type 1 strain. In neutralization tests (Fig. 1) with F, a prototype 1 strain, and G, a prototype 2 strain, MP appears to be antigenically intermediate between the two^{2,7,8,14}. Some variability in the immunological specificity of fresh clinical isolates has also been reported^{6,19}. Type 1 strains are also more heterogeneous with respect to physical properties than type 2 strains²⁰, and, in the newborn, manifestations of type 2 virus are variable whereas in older individuals there is more variability with type 1 infections, probably as a result of the mode of acquisition of the virus infection.

(2) Herpes simplex seems to be very variable in the laboratory. In the past 10 years numerous mutants

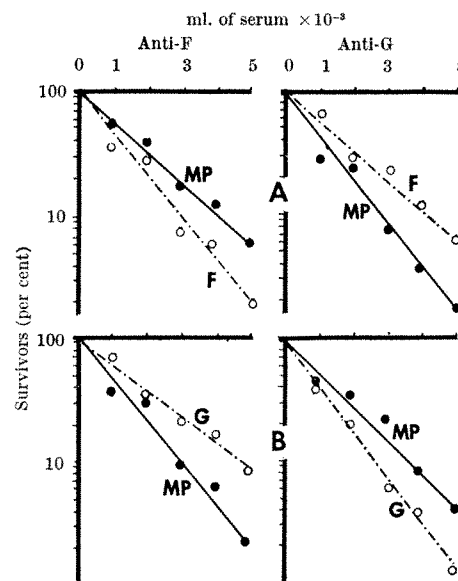


Fig. 1. Differentiation among F, MP, and G strains of herpes simplex virus. A, Neutralization of artificial mixture of F and MP strains with anti-F and anti-G rabbit sera; B, neutralization of artificial mixture of G and MP strains with anti-F and anti-G rabbit sera. The procedure used in these neutralization tests has been described elsewhere^{7,17}.

differing in surface properties and in their effects on cells have been described^{9,17,21,22}. Of considerable weight in our arguments are the studies by Hampar and Burroughs²³ who isolated a spectrum of mutants differing with respect to virulence, ability to multiply in Chinese hamster cells, and in immunological specificity. A similar spectrum of mutants, ranging from a parent incapable of growing in DK cells to progeny differing in surface properties but capable of multiplying in these cells, was also reported¹⁵. Variation in immunological specificity of viruses isolated from successive recurrences of herpetic eruptions has also been described²⁴.

(3) The basis for the high variability of the surface of the herpes simplex virion might be the nature of the envelope of the virus. Data from two laboratories (unpublished work of O. O. Ishevsky, Y. B., J. M. K., P. G. S., R. B. and ref. 25) show that the envelope contains glycoproteins made after infection and inserted into a matrix of preformed lipids. Electropherograms of the glycoproteins (labelled with ³H or ¹⁴C-glucosamine) from partially purified virions of strains differing in surface properties (Fig. 2) are very revealing. Strain F is immunologically type 1. MP, as pointed out earlier, is antigenically intermediate between type 1 (F) and type 2 (G) strains. As shown in Fig. 2, the electropherogram of glycoproteins from the F strain differs substantially from that of the G strain. In several details, the electropherograms of the MP strain is intermediate between those of type 1 and type 2 viruses. Data from our laboratory show that the extent of glycosylation of the proteins is determined in part by the host (unpublished work of J. M. K., P. G. S. and R. B.). It is conceivable that immunological specificity and the other covariant properties cited earlier are determined by the polysaccharide moiety of the glycoproteins, that glycosylation is determined by the primary structure of the envelope proteins, that minute mutations in the envelope proteins drastically effect the nature and extent of glycosylation and that the variety of available mutants and strains reflects selection of amino-acid sequences in the envelope proteins compatible with glycosylating enzymes in the host from which they are derived.

Our thesis is that herpesvirus hominis is not a suitable name for viruses associated with herpes simplex and that,

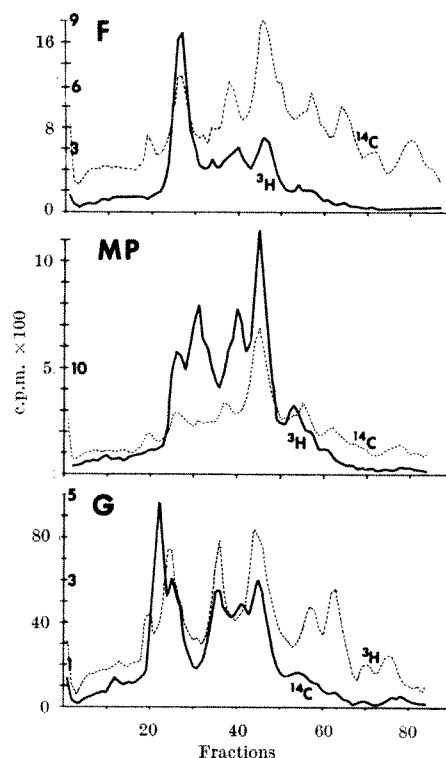


Fig. 2. The electropherograms of the structural proteins of F, MP and G strains of herpes simplex virus. The viruses were grown in HEP-2 cells. The infected cells were overlaid with mixture 199 containing 1/10 the usual amount of leucine, isoleucine and valine. The cells infected with F and MP virus were labelled between 4 and 22 h post infection with ^3H -glucosamine and between 6 and 22 h post infection with ^{14}C -leucine, isoleucine and valine of equal specific activity. For convenience the isotopes were reversed in cells infected with G strain; the reversal did not affect the results. The procedure for preparation of partially purified virus described elsewhere (unpublished work of J. M. K., P. G. S. and B. R.). The procedures for the solubilization of proteins, acrylamide gel electrophoresis and scintillation counting are also described elsewhere²⁵. —, Labelled glucosamine; ---, labelled amino acids. The migration of proteins is from left to right.

in spite of the important differences in their clinical and epidemiological behaviour and the reported differences in the buoyant densities of their DNAs²⁶, the genetic differences between types 1 and 2 viruses are minor and breached by multistep mutations in the laboratory. Specifically, the term herpesvirus hominis means the herpes virus of man. The term implies one of two things. Either man has only one herpesvirus or the virus with that designation is restricted to man alone. In fact, neither of these implications is correct. There are now several recognized herpes viruses infecting man in addition to the virus causing herpes simplex. These are the herpes viruses associated with African lymphoma of children and probably with infectious mononucleosis^{27,28}, the virus of varicella-herpes zoster, and the virus of cytomegalic inclusion disease. Of these viruses, herpes simplex has the widest host range. In fact an epidemic caused by herpes simplex virus has occurred in a monkey colony²⁹. We propose that the classification of herpes viruses based on association with a particular host should be avoided as being misleading and that the herpes virus associated with herpes simplex should continue to be known by that name until a definitive nomenclature is accepted for all herpes viruses. Most of the differences between the so-called type 1 and type 2 strains could be reflexions of the difference in the glycoproteins specified by the virus. While the term "type" is inappropriate for reasons stated earlier, some method of distinction should be used to record the fact that, either because of the site of infection or the method of transmission, relatively stable multistep mutants have been selected and are maintained in nature. We propose to continue the classi-

fication of herpes simplex virus on the basis of serological characteristics until a definitive catalogue of the properties of herpes viruses becomes available. Accordingly, current types 1 and 2 should be referred to as subtypes 1 and 2. Moreover, to integrate laboratory strains into a single classification with fresh clinical isolates we propose that strains intermediate between subtypes 1 and 2, such as MP strain, be referred to as subtype 1/2. The proposed scheme does not obviate the possibility that, ultimately, the minor variations in strains of the two subtypes may turn out to be significant with respect to biochemical characteristics or the illnesses they produce, in which case a new classification may be required.

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Resistance of Mice and Rats to Challenge by *Babesia rodhaini* after Inoculation with Irradiated Red Cells infected with *B. rodhaini*

FIELD vaccination of cattle against babesia parasites is performed by infecting with a strain of low virulence¹. This unfortunately ensures continued transmission of the disease. Some resistance has been induced experimentally in rats^{2,3} and cattle⁴ by injection of killed piroplasms using large amounts of parasite antigen, often combined with an adjuvant. I present here a preliminary account of successful protective immunization of mice and rats against the piroplasm *Babesia rodhaini* using irradiated piroplasms.

Parkes mice and Sprague-Dawley rats were used. The rat-adapted strain of *B. rodhaini*, which is easily transferable to mice, was not contaminated with *Eperythrozoon coccoides*⁵. A stabilate⁶ of infected blood was frozen to -70° C at the start of each experiment to ensure that a strict homologous challenge of vaccinated animals was subsequently made.

Blood was taken from uninfected or infected donors by cardiac puncture, using heparin (17 IU/ml.) as the anticoagulant, and held on ice until irradiated at 22° ± 2° C with approximately 1,000 rad/min from a γ -beam ⁶⁰Co 650 source. Immunizing and challenge inocula were given intraperitoneally. Parasitaemias were determined by examination of Giemsa-stained films of peripheral blood and recorded as the number of parasitized red cells in 10⁵ red cells.

Groups of five or six mice were injected with 2 × 10⁹ infected mouse red cells which had been irradiated with doses of 5, 10, 20, 40 or 80 krad. During the succeeding 20 days recipient mice were examined for piroplasms. As Table 1 shows, only doses of 40 or 80 krad rendered the piroplasms non-infective. On day 20 after immunization the mice in the latter two groups, together with control mice which had been injected with irradiated normal red cells, were challenged with 1 × 10⁶ parasitized red cells. Comparison of the geometrical mean parasitaemias for each group following challenge showed that the mice inoculated with irradiated piroplasms developed patent parasitaemias 3–4 days after the controls and more of them survived.

Table 1. INFECTIVITY OF IRRADIATED MOUSE RED CELLS PARASITIZED BY *Babesia rodhaini*

Radiation received by infected red cells (Krad)	No. of mice which developed an infection after receiving irradiated blood	No. of infected mice which died		
		No.	Day of death Mean	Range
5	5/5	5/5	6	5–7
10	5/5	5/5	8.4	7–10
20	5/5	3/6	16.6	16–18
40	0/5			
80	0/6			

Other experiments with mice were carried out with essentially similar results.

Protective immunization of rats by inoculations of irradiated *B. rodhaini* infected red cells was much more effective than that of mice. 60–80 g rats were inoculated with 1–2 × 10⁹ infected rat red cells which had been inactivated with 60 krad. The results of a typical experiment are given in Fig. 1. Not only was the patent parasitaemia following challenge delayed by about 8 days in the immunized rats but it was also considerably depressed. A fuller account of these experiments is in preparation.

Although the immunizing inocula contained many parasitized cells the strength of the immunity observed in the immunized rats suggests that smaller immunizing inocula can be expected to be effective at least in this host. This type of approach in the control of economically important piroplasms could be worth while even when taking into account the problems associated with intra-specific⁷ variation.

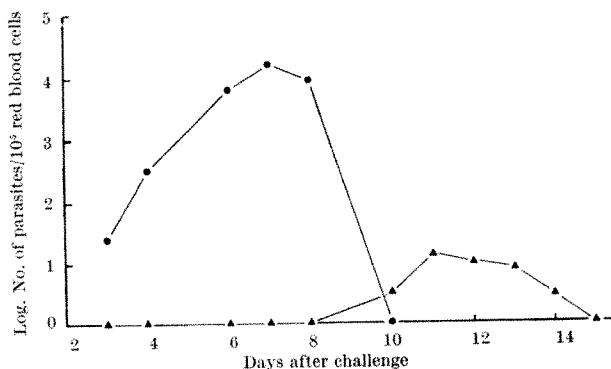


Fig. 1. The immunized rats received 1.5 × 10⁹ rat red cells infected with *B. rodhaini*, and irradiated with 60 krad. Controls (●) received uninfected blood irradiated with 60 krad. Challenge (▲) was with 1 × 10⁶ parasitized rat red cells 19 days after the immunizing inocula. The parasitaemias in the figure are the geometrical means of five rats.

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Renal Prostaglandins: Possible Regulators of the Renal Actions of Pressor Hormones

WE describe here the release from the kidney by angiotensin II and noradrenaline of substances which have the properties of prostaglandins. This observation may help to account for the antihypertensive function of the kidney first suggested by Fasciolo¹, who demonstrated that removal of the kidney contralateral to an ischaemic kidney produced sustained hypertension in dogs. We have adapted the bioassay method of Vane², the superfused blood-bathed organ technique, for the detection of prostaglandins in renal venous blood in response to infusions of angiotensin II or noradrenaline into the renal artery.

Male mongrel dogs (22–35 kg) were anaesthetized with morphine sulphate (2 mg/kg, subcutaneously) and chloralose (100 mg/kg, intravenously). Anaesthesia was maintained with chloralose (40 mg/kg). The trachea was cannulated and the lungs were ventilated mechanically. The abdominal cavity was opened by a transverse incision and the right renal artery and right ureter were isolated. Two Hewlett-Packard direct writers recorded: (1) mean aortic blood pressure measured by a Statham transducer ('P23Db'); (2) renal blood flow measured by an electromagnetic flowmeter ('Statham M-4001'); (3) changes in length of assay organs as detected by Harvard isotonic transducers; and (4) urine drops counted by a Grass photoelectric transducer ('PTTI'). Rat stomach strip, rat colon and chick rectum, which were reported by Ferreira and Vane³ to possess the required specificity and sensitivity for the detection of prostaglandins were used as assay organs. The assay tissues were loaded with 1 to 3

g and suspended in polypropylene chambers from auxotonic levers⁴. Heparin (1,500 IU/kg) and dextran of 70,000 average molecular weight (10 ml/kg) were given intravenously just before superfusing the assay tissues. Renal venous blood was withdrawn by a pump at 15 ml/min and returned to the dog through the left jugular vein. Either angiotensin II or noradrenaline dissolved in 0.9 per cent saline was infused into the right renal artery. Substances appearing in renal venous blood during infusion of the pressor hormones, which affected the activity of these assay organs, were tentatively identified and their concentrations were estimated by matching infusions of standard solutions delivered into the extracorporeal circuit.

In three experiments with the appearance of prostaglandin-like activity, as detected by the assay organs, 300 to 500 ml. of renal venous blood were collected in ethanol and were extracted for acidic and neutral lipids with chloroform according to procedures described previously⁵ which eliminate all substances known to affect these assay organs except acidic lipids^{3,6}. Dextran was infused at the same rate as the venous blood was removed. The acidic lipids were further purified by thin-layer chromatography⁷ on silica gel layers (0.5 mm thick), using the solvent system (chloroform-methanol-acetic acid; 18:1:1, by volume) described by Daniels *et al.*⁸. The lipid extracts and the eluates from thin-layer chromatographic zones were reconstituted in saline for bioassay on the organs superfused with Krebs solution. Their effect on assay organs was compared with that produced by prostaglandin E₂ standards.

Angiotensin II or noradrenaline, when infused into the renal artery, decreased urine flow and renal blood flow, but in spite of continued infusion of angiotensin II or noradrenaline, coincident with release of prostaglandin-like substances into renal venous blood, renal blood flow

and urine flow returned towards control values (Fig. 1). In nine experiments, doses of angiotensin II (0.5 to 20.0 ng/kg/min) which released prostaglandin-like substances reduced renal blood flow from 12 to 78 per cent of control values (mean reduction 35 per cent). The threshold dose of angiotensin II in arterial blood required to release prostaglandin-like substances varied from 0.1 to 4.4 ng/ml. (mean 1.0 ng/ml.). This release was non-specific because equiconstrictor doses (1 to 100 ng/kg/min) of noradrenaline also released them in two of five experiments. Concentrations of prostaglandin-like substances in renal venous blood, when assayed as prostaglandin E₂ equivalents, ranged from 0.1 to 3.3 ng/ml. (mean of fifteen observations, 0.9 ng/ml.). Their concentration was unrelated to the dose of either hormone or the degree of renal vasoconstriction.

Lipid extracts of renal venous blood obtained during control periods did not contract the assay organs, except in one experiment when a prostaglandin E-like material was recovered in a concentration less than one-fifth those obtained during experimental periods. Extracts of renal venous blood obtained during infusions of the pressor hormones and samples obtained from prostaglandin E zones of the chromatographed extract showed activity similar to prostaglandin E₂ standards (Fig. 2). Differences in the ratio of activities of the assay tissues in response to prostaglandin E₂ standards and extracts of renal venous blood might be accounted for by the presence of an F prostaglandin. The definitive identification of this material as prostaglandin E₂ has yet to be established. Thus final characterization of the prostaglandin-like substances by argentous thin-layer chromatography was not possible because of insufficient material.

The antihypertensive function of the kidney may depend on the release by angiotensin II of renal prostaglandins E₂ or A₂ which are vasodilator depressor sub-

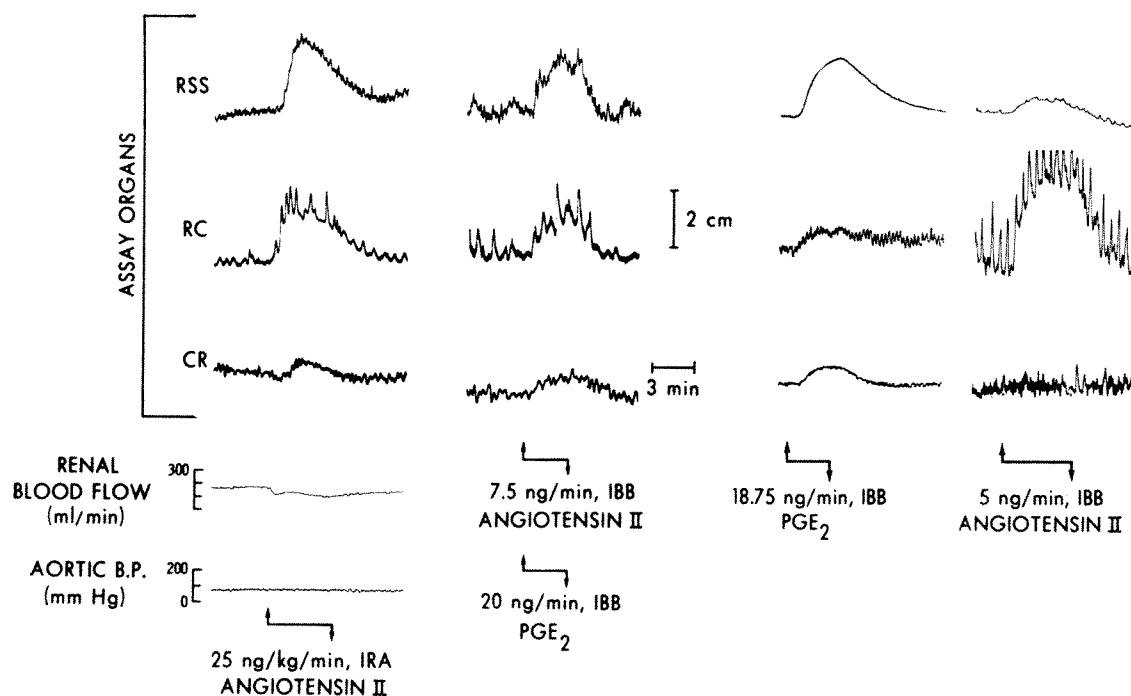


Fig. 1. Effects of angiotensin II infused into the renal artery (IRA) on mean aortic blood pressure (BP), renal blood flow and assay organs superfused by venous blood of the same kidney in a chloralose-anaesthetized dog. Angiotensin II and PGE₂ (prostaglandin E₂) standards, simultaneously infused into the extracorporeal circuit (IBB), matched the contractions of the assay organs produced by infusion of angiotensin II IRA. Infusions of either standard IBB did not reproduce the effects on assay tissues of angiotensin II given effluent of some of the infused angiotensin II simultaneously with prostaglandin-like substances presumably released by angiotensin II. Renal blood flow was reduced from a control value of 175 to 116 ml/min as an immediate response to angiotensin II infusion. Coincident with the appearance of prostaglandin-like substances as detected by the assay organs, renal blood flow increased to 134 ml/min. 90 min elapsed between the sections of the tracings on the left. The responses of the assay organs shown in the tracings on the right were obtained during superfusion with Krebs solution. RSS, rat stomach strip, RC, rat colon, CR, chick rectum. Time scale, 3 min; vertical scales: 2 cm for assay organ activity, renal blood flows in ml/min, aortic BP in mm Hg.

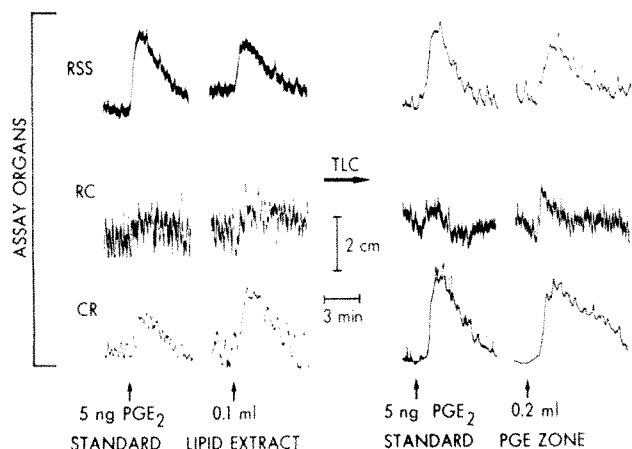


Fig. 2. Renal venous blood, which was obtained when prostaglandin-like activity of the assay organs occurred during infusion of angiotensin II, was extracted for acidic and neutral lipids and the extract purified by thin-layer chromatography (TLC). The effects on assay organs of samples of PGE (prostaglandin E)-like material isolated from blood were compared with those of standard solutions of PGE₂. The chromatographic and biological properties of the extracted and purified prostaglandin-like substances are similar to PGE₂ standards. RSS, rat stomach strip, RC, rat colon, CR, chick rectum. Time scale, 3 min; vertical scale, 2 cm.

stances⁹ present in canine kidney¹⁰. The threshold concentrations of angiotensin II required to release prostaglandin-like substances were in the range of those reported in renal vascular hypertension (0.04 to 0.41 ng/ml. of blood)¹¹, but 90 to 95 per cent of circulating prostaglandin E₂ is removed by the lung³ in contrast to the free passage across the lung of prostaglandin A₂ (ref. 9). We cannot detect the release of prostaglandin A₂ because the bioassay procedures are relatively insensitive to prostaglandins of the A series. Blunting of the renal vasoconstrictor and antidiuretic actions of angiotensin II (refs. 12 and 13) or noradrenaline may depend on release of renal prostaglandins. Prostaglandins of the E series increase renal blood flow, urine flow and sodium excretion^{9,14} and inhibit the effects of angiotensin II on renal function¹⁵. The threshold dose of prostaglandin E₂ required to increase renal blood flow and urine flow (0.1 ng/ml. of blood)⁹ is one-ninth that of the estimated concentration of prostaglandins assayed as prostaglandin E₂ appearing in renal venous blood in response to either pressor hormone. In several experiments, when release of prostaglandin-like substances was not observed, recovery of renal blood flow and urine flow did not occur.

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Accumulation of Chorio-retinotoxic Drugs in the Foetal Eye

USING whole body autoradiography¹ we have found a selective accumulation and retention of certain drugs in the eyes of mouse fetuses. These drugs are known to cause ocular damage in adult humans and experimental animals. The injury (toxic retinopathy), which is not seen in albino animals, has been related to the affinity of the drugs for the melanin structures of the eye². The risk of foetal ocular damage appears to have been neglected in the past.

Different categories of drugs cause ocular damage through their affinity to the pigment. Severe retinal damage, including blindness, was first observed during clinical tests of the phenothiazine derivative NP-207 (ref. 3). Similar damage, although less pronounced, has been reported after clinical use of other phenothiazine drugs, such as thioridazine and chlorpromazine⁴, which are frequently used as antiemetics and tranquilizers during pregnancy. Severe retinopathy is also reported after use of the antirheumatic compounds chloroquine⁵ and indomethacin⁶ and the cytotoxic agent sparsomycin⁷.

The clinical picture in toxic retinopathy is characterized chiefly by retinal pigmentation, visual field defects and reduced visual acuity. The retinal pigmentation is due to a migration of pigment granules into the receptor layer of the retina from the degenerated retinal pigment epithelium⁸. In addition a pigmentation in the cornea and the lens has been reported as a side effect during long-term therapeutic use of phenothiazines, especially chlorpromazine⁹. This pigmentation, however, has not been related to the affinity of the drugs for melanin.

We have administered ³⁵S-chlorpromazine or ¹⁴C-chloroquine intravenously to pregnant pigmented CBA mice. Whole body autoradiography was then performed after varying intervals following injection on either the whole pregnant mouse or of mouse fetuses which had been removed surgically from injected mothers (Fig. 1).

Both drugs passed rapidly across the placenta and accumulated in the eyes of both fetuses and mothers. Pronounced radioactivity remained in the ocular tissues for 5 months after the drugs had been eliminated from other tissues.

We have also investigated the affinity of chloroquine for various tissues in sections through uninjected mice. Whole body sections were immersed in a water solution containing ¹⁴C-chloroquine, washed, dried and autoradiographed. There was selective uptake of ¹⁴C-chloroquine into the melanin-containing structures where intravenously injected ¹⁴C-chloroquine had accumulated in the living animal (Fig. 2).

The cause of this ocular damage is not fully understood. Charge transfer¹⁰ has been suggested as the

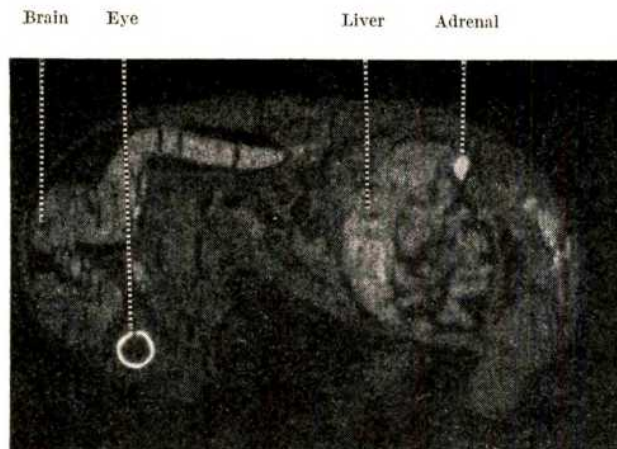


Fig. 1. Autoradiogram of a pigmented mouse foetus 1 h after intravenous injection of ^{35}S -chlorpromazine to the mother. The foetus was removed by cesarian section. Note the marked accumulation in the eye.

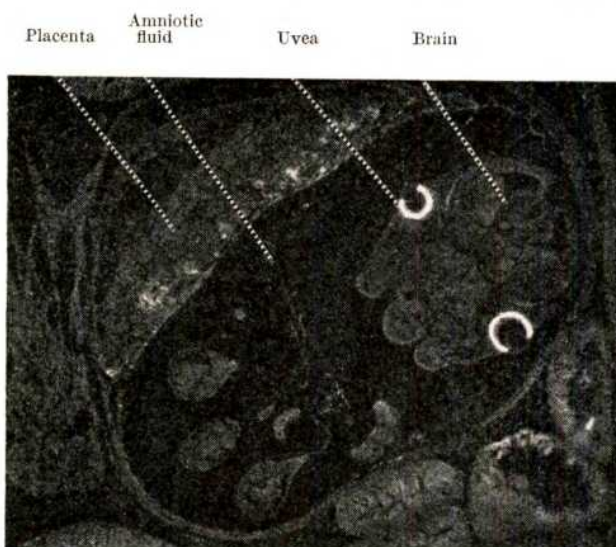


Fig. 2. Detail of an autoradiogram of a pregnant pigmented mouse showing the *in vitro* affinity of ^{14}C -chloroquine for foetal uveal melanin.

mechanism for the accumulation of drugs in melanin. Retinal damage always seems to be secondary to the accumulation of drug in the pigment. Perhaps drug-induced pigmentation of the cornea and the lens is caused by fragments of melanin released from the iris into the aqueous humor. The pigmentation is found on the anterior capsule of the lens and on the posterior surface of the cornea.

It is very difficult to estimate the risk of foetal ocular damage in connexion with the clinical use of these drugs during pregnancy. But the strong affinity observed the early development of the foetal pigment epithelium and the often relatively great sensitivity of foetal tissues compared with those of the adult, indicate that there is a risk which should not be overlooked. If damage has occurred which reduces vision, it may not be discovered until long after birth and may then be difficult to relate to medication during pregnancy.

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Molar Absorbance Coefficient of Rhodopsin

UNTIL recently, it was accepted that the molar absorbance coefficient of rhodopsin, ϵ_{rh} , was no less than 41,000 (refs. 1-4). In 1968, however, Heller⁵ obtained a value of 23,100 by measuring the amount of protein in purified samples of rhodopsin and assuming a protein molecular weight of 26,400.

One of the earlier methods¹ had depended on measurement of the absorbance of retinal oxime produced when a solution of rhodopsin containing hydroxylamine was bleached. From the molar absorbance coefficient of retinal oxime, the corresponding value for rhodopsin was calculated. In private correspondence with me, Heller suggested that unspecified interactions with opsin in the bleached extract might reduce the absorption of retinal oxime, so yielding a spuriously high ϵ_{rh} . This seemed unlikely, for the absorption spectrum of retinal oxime is normal in such extracts¹. I have, however, re-evaluated the oxime method in a new series of observations on rhodopsin extracted with digitonin and cetyltrimethylammonium bromide (CTAB, the extractant used by Heller)⁶. Contrary to the above suggestion, opsin and other proteins had no effect on the absorption of retinal oxime, and the mean value of ϵ_{rh} in digitonin and CTAB was found to be 41,900 (ref. 6).

By determining retinal colorimetrically⁷ in solutions of bleached rhodopsin, Shichi *et al.*⁴ found an ϵ_{rh} of 42,000 in a third extractant, 'Emulphogene BC-720'.

Following our correspondence, Heller⁸ redetermined ϵ_{rh} by his previous method, and has now reported 21,524, slightly lower than the earlier result. Although Shichi *et al.* had reported a reproducibility of $\pm 1-5$ per cent in their retinal determinations, Heller claimed that in his laboratory their technique gave "only very erratic and non-reproducible results", leading to a low value for the amount of retinal and therefore a high ϵ_{rh} . The effect was attributed to competition for retinal by amino groups on the opsin, and perhaps losses by oxidation or reduction. Heller⁸ then continued: "similar considerations may apply to the oxime method". The purpose of my communication is to show that this proposition is unacceptable, and that at present there is no plausible explanation for the discrepancy between the different methods.

When visual pigments are bleached in the presence of 10-20 mM hydroxylamine, the change of absorbance resulting from the formation of retinal oxime is constant, irrespective of whether the pH is 6 or 9. This does not support the notion that amino groups compete with hydroxylamine, for appreciable condensation should occur at alkaline pH (producing Schiff's bases) but virtually none in mildly acid conditions. The following simple experiment illustrates the point directly.

A solution of frog opsin in 2 per cent digitonin was divided into two portions which were then buffered to pH 7 and 9, respectively. A 1 ml. aliquot of each was mixed with 1 ml. of all-*trans* retinal in digitonin. The final protein absorbance at 275 nm was about 0.9. A "free retinal"

solution was made by substituting 1 ml. of digitonin at pH 7 for the opsin. After incubation at 20° C for 0.8 h, the λ_{\max} of the neutral preparation was 387 nm, in the same position as that of the "free retinal" solution, whereas that of the alkaline preparation was 373 nm. This λ_{\max} is close to that of the Schiff's base. Hydroxylamine was then added to each of the three mixtures (final concentration 10 mM). The absorption spectra shifted until their λ_{\max} values were at 367 nm, typical of retinal oxime. After a small correction (about 0.03) had been made for the absorption of the opsin, the absorbances at this wavelength were 0.350, 0.352 and 0.342 for the neutral opsin, alkaline opsin and "free retinal", respectively. Thus it is clear that oxime formation is not hindered by the presence of opsin.

With regards to Heller's second suggestion, losses by oxidation or reduction of retinal would have to amount to nearly 50 per cent, and should be easily detected by spectroscopic means.

In conclusion, it should be noted that Heller's ϵ_{rh} would be raised to 43,000 if his molecular weight were doubled to 53,000: an improbable figure, however, because both Shields *et al.*⁹ and Shichi *et al.*⁴ agree on a molecular weight near 28,000. Yet this finding merely increases our difficulties, for both groups^{4,9} base their calculations on the ϵ_{rh} of 41,000–42,000 obtained by the "oxime" and "retinal" methods.

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Molar Absorbance of Cattle Rhodopsin

An accurate value for the molar absorbance of the visual pigment rhodopsin is important in quantitative studies on the biochemistry of vision. A recent controversy regarding this value^{1,2} prompted the present study.

The first value for the molar absorbance of cattle rhodopsin was reported by Wald and Brown³. They illuminated a rhodopsin sample of known absorbance in the presence of hydroxylamine to obtain quantitatively the all-*trans* retinaldehyde in the form of its oxime. Having determined the molar absorbance of pure all-*trans* retinylidene oxime at 360 nm, they calculated for rhodopsin a molar absorbance of 40,600 from the ratio of absorbance at 500 nm before and at 360 nm after photolysis. They assumed that one retinaldehyde was present per rhodopsin molecule, an assumption which was subsequently proved to be correct⁴. Futterman and Saslaw⁵ confirmed the value of Wald and Brown by determining retinaldehyde in retina and in rod outer segment preparations by means of their thiobarbituric acid method. Recently Heller⁶ reported a much lower value of $23,100 \pm 800$, which he calculated from the absorbance at 500 nm of a rhodopsin sample, its amino-acid composition and a molecular weight for the protein part of 26,400, assuming that the preparation

was pure rhodopsin. Subsequently Shichi *et al.*² arrived at a value of 42,000 from the absorbance of purified rhodopsin and a retinaldehyde determination in the same sample by the thiobarbituric acid method and Shichi⁶ now confirms this value by means of the method of Wald and Brown³. Heller⁷ replied that the thiobarbituric acid method gave "only very erratic and non-reproducible results with visual pigments, though excellent reproducibility and accuracy were obtained with free all-*trans* retinaldehyde". We have examined the problem by means of a slightly modified form of the method of Futterman and Saslaw⁵.

All rod outer segment preparations were isolated as described recently⁸. Rhodopsin was determined in buffered 1 per cent Triton 'X-100' solution (pH 6.5) from the difference in absorbance at 500 nm before and after photolysis (illumination by 75 W tungsten lamp at a distance of 20 cm; ultraviolet and infrared filters; 10 min) in the presence of 0.05 M hydroxylamine. The decrease in absorbance at 500 nm for solutions containing 1 mg of the dry rod outer segment preparation per ml. solution is defined by us as the specific absorbance difference (ΔA_{500}). Normally it varied between 0.150 and 0.250 in our outer segment preparations.

Retinaldehyde was determined as follows. Samples of lyophilized outer segments (1–2 mg) were weighed on a torsion balance (accuracy 0.02 mg) and placed in Potter-Elvehjem tubes, which could be centrifuged. The material was homogenized in 1 ml. of a freshly prepared thiobarbituric acid-thiourea mixture⁵, to which 100 μ l. propanol was added. The mixture was left in darkness for 30 min, homogenized a second time and centrifuged. The absorbance of the supernatant was measured at 530 nm. The assay was standardized against pure all-*trans* retinaldehyde in propanol (0.1 mM).

In the original method of Futterman and Saslaw⁵, retinaldehyde was extracted with 90 per cent ethanol, and was thereupon determined in an aliquot of this extract. We find that pre-treatment with ethanol is not essential, for the thiobarbituric acid-thiourea reagent extracts the retinaldehyde from the pigment material equally well. In three different outer segment preparations the direct method gave 1.05 (s.e. 0.05) times the value obtained by the original method. The principal advantage of the direct method is that one-sixth of the amount of retinaldehyde can be determined with reasonable accuracy. Furthermore, the number of manipulations is decreased, because the direct extraction of retinaldehyde with the reagent obviates the need for extraction of rhodopsin into detergent solution and of retinaldehyde into aqueous ethanol.

The recovery of retinaldehyde added to rhodopsin was also checked, for Heller⁷ suggested that the variability and low yield of the thiobarbituric acid reaction might arise from a reaction of retinaldehyde with amino groups of the protein component of rhodopsin. Known amounts of retinaldehyde in propanol were pipetted into the Potter-Elvehjem tubes, a weighed sample of outer segment preparation was added and the assay was carried out as described. The recovery of added retinaldehyde was 103 per cent (s.e. 4 per cent; $n=7$).

The correlation between specific absorbance difference (ΔA_{500}) and retinaldehyde content of twenty-three different rod outer segment preparations is illustrated in Fig. 1. The correlation coefficient is 0.90 ($P<0.001$). The intercept of the regression line ($y=0.022+0.135x$) is not significantly different from zero (Student intercept $t_{21}=1.10$; $P>0.25$). So a straight proportionality exists between the ΔA_{500} and the retinaldehyde content of the outer segments. This is further illustrated by the four points in the lower left of the figure, which represent values for whole retina and which fall close to the line. Because a 1:1 molar ratio of retinaldehyde to rhodopsin has been established⁴, the molar absorbance of rhodopsin can be calculated from the average ΔA_{500} and retinaldehyde

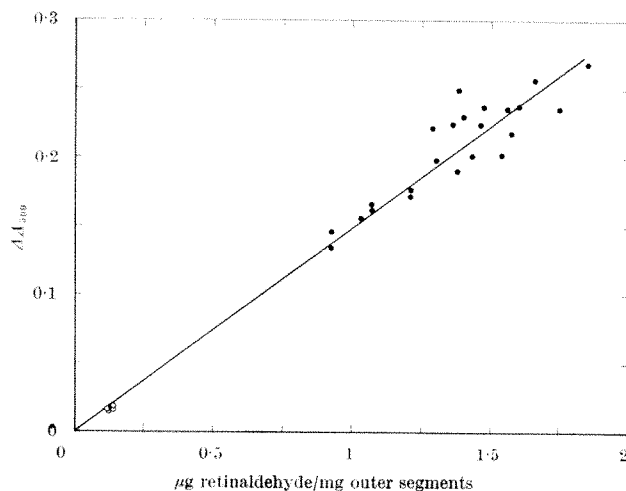


Fig. 1. Correlation between ΔA_{400} and retinaldehyde content of twenty-three different cattle rod outer segment preparations. The four isolated points in the lower left part represent values obtained from whole homogenized, lyophilized cattle retinas.

content. From our results for rod outer segment preparations of high purity, the molar absorbance of cattle rhodopsin is calculated to be 43,000 mole/cm² (s.e. 700; $n = 23$).

All retinaldehyde determinations by the thiobarbituric acid method have been standardized against all-*trans* retinaldehyde. In native rhodopsin, however, retinaldehyde is in the 11-*cis* configuration. In earlier reports it is not always clear whether or not the rhodopsin preparations had been subjected to photolysis before the retinaldehyde assay. This might cause an error in the molar absorbance value, if all-*trans* and 11-*cis* retinaldehyde have a different chromogenicity. Although we found the same results, regardless of whether all procedures including the retinaldehyde assay were carried out in dim red light or in normal room light, we have explicitly tested this possibility by comparing the chromogenicity in the thiobarbituric acid assay of rhodopsin and metarhodopsin I samples obtained from the same outer segment preparation. Metarhodopsin I, which contains all-*trans* retinaldehyde as chromophoric group, was prepared by illuminating rod outer segments in the dry state for 30 min with intense visible light from which ultraviolet and infrared radiation was filtered. Metarhodopsin I formation was indicated by a colour change from purple to orange. All other manipulations were carried out in dim red light to prevent other light-induced isomerizations. For four different outer segment preparations the chromogenicity in the thiobarbituric acid assay of the samples in the metarhodopsin I state was 97.4 per cent (s.e. 2.6) of that in the rhodopsin state. In agreement with this observation, chromatographically pure 11-*cis* retinaldehyde⁹ (prepared from 11-*cis* retinoyl acetate) produced a chromogen in the thiobarbituric acid assay with absorption maximum and molar absorbance value identical to those of all-*trans* retinaldehyde. Thus we conclude that all-*trans* and 11-*cis* retinaldehyde show equal chromogenicity in the thiobarbituric acid method, as previously suggested by Futterman and Saslaw⁵.

Our result agrees within the experimental error to the findings of Wald and Brown³ and Shichi *et al.*² and we do not confirm the much lower value for the molar absorbance of rhodopsin of Heller¹.

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Specific Ion Electrode in the Determination of Urinary Fluoride

THE introduction of a fluoride sensitive specific electrode¹ has greatly simplified the determination of fluoride ion and recently its use has been proposed for the determination of urinary fluoride²⁻⁵. This article draws attention to an interesting and important anomaly which has been observed in using the electrode for the routine screening of workers engaged in the manufacture of hydrofluoric acid.

Samples of urine were taken at random from male adults. Direct measurements of fluoride were made with the Orion electrode (model 94-09) in conjunction with the Orion specific ion meter (model 401). Microdiffusions and spectrophotometric determinations of fluoride were made by the methods of Hall^{6,7}.

The following procedures were adopted for both urine specimens and fluoride standards. (1) The urine was diluted with (a) a molar trisodium acetate solution (1 + 9 ml.) adjusted to pH 5.4; (b) Orion total ionic strength acetate buffer (TISAB) (ref. 8), (1 + 7 ml.). Direct fluoride measurements were then made with the electrode. (2) The urine was diluted with 0.4 M H₂SO₄ (1 + 9 ml.) and 0.5 g Ag₂SO₄ added to the mixture to precipitate chloride ions. Fluoride was then diffused from 1 ml. portions of the chloride-free solution and determined (a) spectrophotometrically and (b) with the electrode after extraction into an acetate buffer of pH 5.2. (3) The urine was mixed with an equal volume of molar perchloric acid and (a) placed in a boiling water bath for 15 min, or (b) left at ambient temperature (18°–22° C) for 15 min. After cooling the tubes containing the heated urine, the mixtures were diluted with three volumes of 15 per cent trisodium acetate solution (final pH 5–5.5) and fluoride was determined with the electrode. Measurements were made at least in triplicate.

The results (Table 1) show that these urines contained a form of fluorine which was not measured directly by the

Table 1. COMPARISON OF URINARY FLUORIDE DETERMINATIONS

	Electrode (direct)		Microdiffusion		HClO ₄ with electrode	
	Na acetate buffer (1a)	TISAB (1b)	Alizarin complexan (2a)	Electrode (2b)	100° C (3a)	18°–22° C (3b)
1	11.4	14.3	21.0	18.6	20.5	13.6
2	17.1	21.8	39.2	39.9	37.0	21.7
3	19.2	22.9	45.2	44.8	40.0	24.2
4	7.2	6.8	14.8	14.9	14.2	8.4
5	10.2	10.7	16.5	17.0	16.2	10.5
6	11.6	12.5	15.3	14.5	15.8	12.9
7	10.1	10.8	18.4	16.9	18.0	13.2
8	18.6	20.5	25.2	23.9	24.0	19.4
9	11.6	11.1	33.4	27.6	31.2	12.0

Values represent μg of fluoride per ml. of urine.

electrode. The explanation seems to be that some of the fluorine is present in non-ionic form, but can be changed by hot, dilute perchloric acid or during diffusion. In four examples, the fluoride measured directly by the electrode was less than half of that following diffusion or treatment with hot 0.5 M perchloric acid.

The values obtained after microdiffusion are in close agreement when measured either by the electrode or spectrophotometrically and reflected more realistically the true fluoride levels. The results with the cold perchloric acid preclude the possibility that the difference between the direct electrode readings and those following diffusion of the fluoride could be due to a relatively insoluble compound such as calcium or magnesium fluoride.

The observation that there can be circumstances when the electrode may not measure all the fluoride present in the urine is very important in its application in clinical medicine. Fortunately, it seems that pre-treatment with perchloric acid affords a simple means to obtain with the electrode a more accurate direct assessment of urinary fluoride.

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Hypnotic Susceptibility increased by EEG Alpha Training

MORE than a decade of research on hypnotic susceptibility and its correlates has demonstrated repeatedly the stability of the phenomenon, its independence from standard tests of suggestibility and of personality, and its relative imperviousness to most efforts to manipulate it, in particular to increase it^{1,2}. Several recent studies have shown, however, that hypnotic susceptibility is significantly correlated with brain wave activity, including the duration of electroencephalogram (EEG) alpha rhythms³⁻⁵. Other studies have shown that the production of alpha rhythms can be systematically increased by teaching subjects to accommodate their subjective mental states to sustained auditory feedback signals which indicate the continuous manifestation of this rhythm^{6,7}.

We have investigated the relationship between hypnotic susceptibility and brainwave activity, on the hypothesis that successful training to increase alpha rhythm duration would raise hypnotic susceptibility.

Thirty subjects (10 male and 20 female), 17 to 62 yr old, were selected from 180 volunteers recruited through local newspapers. They were first screened on the Harvard

group scale of hypnotic susceptibility (HGSS)⁸, administered on a tape recording, and those with scores of 7 or below were scheduled for EEG measurements and individual testing on form A of the Stanford hypnotic susceptibility scale (SHSS)⁹. Subjects with base-rate alpha production of 2 min or less from the total time of measurement of 4 min (2 min eyes open, 2 min eyes closed) and with an average score on the group and individual hypnotic susceptibility scales of 7 or less, then received six training sessions in 1-2 weeks.

The sites where electrodes were placed for all screening and training EEG measurements were mapped according to the international 10-20 system. The four recorded channels were: channel 1, Fp2-LEar; channel 2, Fp1-LEar; channel 3, Fp2-LEar; channel 4, O₁-LEar. Channel 1 was selected for online monitoring and training of EEG alpha; the frontal electrode site was chosen, instead of the usual occipital site for alpha feedback, to set a stringent criterion for the alteration of alpha levels⁶.

The EEG recording and training system consisted of: (a) a Grass model 7 four channel polygraph with P1 preamplifiers; (b) a Krone-Hite model 330-A bandpass filter set at 10 Hz, connected to the polygraph by a lead from J, of channel 1 and (c) an amplitude sensitive trigger connected to the amplified output of the filter, set to close for unattenuated alpha signals. Outputs from the trigger activated (d) a Berkeley model 554 Eput Meter for the direct timing of alpha durations from a 100 Hz precision oscillator within the trigger, (e) a signal marker on the polygraph for visual checking of trigger accuracy, (f) a Mighty-Light photoelectric strobe in the subject room activated by the unadjusted-amplitude EEG signals, and (g) a Heath EUW27 audio oscillator activated by adjusted-amplitude signals leading to (h) a speaker in the subject room. Outputs from the trigger and three channels of the polygraph were connected to (i) an Ampex SP300 tape recorder, and output from (j) a Roberts 1670 internally modified tape recorder connected to a trigger input. Circuit diagrams of the trigger, the only non-commercial device used, are available in ref. 10. This system provided tracking feedback through the strobe light and on-off target feedback through the oscillator tone when the trigger was operated in the feedback mode. Pilot studies indicated that the combined light and tone gave a more powerful training effect than other procedures.

For the training sessions, twenty subjects were assigned at random to an experimental and ten to a control group. All were told that the study involved training in brain wave autocontrol as a method of improving hypnotic susceptibility. The training sessions each consisted of 4 min of base rate EEG measurement and six blocks of 5 min feedback trials, during which all frontal brain waves produced synchronous flashes and alpha waves produced the tone as well. All subjects were told to try to maintain the tone. The feedback tone which control subjects heard, however, was not their own, but was activated by the prerecorded EEG tape of a single subject from the experimental group who had shown marked improvement across the training sessions. At the end of each session, subjects evaluated their feelings during alpha production and/or hypnosis by rating each of forty-eight adjectives describing different mental states¹¹. At the end of the sixth session, a final EEG base rate measurement was recorded and the SHSS form B was administered by an experimenter who had not previously tested the subject⁸. After each control subject finished he was informed that he had seen a control group, and the nature of the feedback he received was explained. He was then offered the opportunity to receive real feedback for two post-experimental sessions.

Results were analysed by correlation techniques and by analysis of variance and covariance. They indicate that hypnotic susceptibility and operant alpha rhythm are positively related both before and after subjects have

received alpha training. Pretraining susceptibility and alpha measures yield product moment correlations of 0.79 ($P < 0.001$); after training 0.65 ($P < 0.001$). Changes in both variables, moreover, correlate 0.73 ($P < 0.001$) with each other. Table 1 shows mean alpha duration scores and hypnotic susceptibility scores before and after training for both subject groups.

Table 1. MEAN BASE RATE ALPHA AND HYPNOTIC SUSCEPTIBILITY SCORES FOR ALL SUBJECTS AND CONDITIONS

Group	Hypnotic susceptibility				Alpha production			
	Pre-training		Post-training		Pre-training		Post-training	
	\bar{X}	s.d.	\bar{X}	s.d.	\bar{X}	s.d.	\bar{X}	s.d.
Contingent Feedback (n=20)	3.30	2.24	7.45	2.56	28.35	16.61	103.60	34.47
Pseudo Feedback (n=10)	4.60	1.86	6.20	2.68	44.40	34.89	82.10	38.97
All subjects (n=30)	3.73	2.21	7.03	2.67	33.70	25.44	96.43	37.43

Analysis of variance of these scores indicates that subjects in both groups increased alpha duration significantly as a result of training ($F = 72.33$, $P < 0.001$), and analysis of covariance, with pretraining alpha scores as the covariates, indicates that the experimental group benefited significantly more from training than did the control group ($F = 4.85$, $P < 0.05$). Similarly, the hypnotic susceptibility of subjects in both groups rose significantly ($F = 37.86$, $P < 0.01$). Covariance analysis with pretraining susceptibility as the covariate, however, indicates that experimental subjects gained significantly more than did controls ($F = 4.97$, $P < 0.05$). Kasamatsu and Hirai found that the EEG alpha of experienced meditators shifted anteriorly while they were meditating⁹.

The subjective descriptions of feelings accompanying alpha and hypnotic induction complement the objective scores. Comparisons of standard scores for all forty-eight descriptive adjectives yielded a correlation of 0.89 ($P < 0.001$) for their application to both experiences, indicating that alpha and hypnosis were typically experienced as very similar subjective states.

The experimental demonstration of a functional relationship between EEG alpha and hypnotic susceptibility challenges widely held theories of hypnosis, especially role playing theories, and supports the idea that hypnosis is a psychophysiological state.

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Inherited Tremor in the Domestic Cat, *Felis catus* L.

THE only reported case of an inherited disturbance of neurological function in cats other than deafness or ataxia resulting from cerebellar hypoplasia¹. Kilhar and Margolis², however, have confirmed suggestions of Verlinde and others³ that most cases, if not all, are of viral origin. The very scanty evidence presented to support the hereditary hypothesis must be considered insufficient in view of the careful documentation of the viral aetiology. Thus, no hereditary locomotor defect has been demonstrated in cats, although many have been described in the mouse⁴ and other animals⁵.

A tom cat (domestic shorthair given to us by Mr E. Warner) mated to one of his daughters produced a litter of three, one of which developed severe trembling, great difficulty in walking and feeding, markedly retarded growth and died early. A repeat of this mating produced a litter with a single living kitten which developed the same symptoms. Since then, this male has been repeatedly mated to his female progeny as they mature. So far thirteen daughters and two granddaughters have been mated with the male and four daughters and one granddaughter have produced affected offspring. A total of eleven affected (six female and five male) and thirty-five normal kittens issued from these matings. Two sons of the tom have been mated with the females that have produced offspring manifesting the trembling. One son produced sixteen normal offspring, the other has produced four affected and twelve normal kittens. The original male has produced many kittens in matings to unrelated females, and none had these symptoms. Of more than 325 litters produced in our colony, these have been the only cases of this trembling and staggering to appear.

The signs of the disease are quite different from the ataxia associated with cerebellar hypoplasia both in structure and in behaviour. Most strikingly, the cerebellar structure is not noticeably abnormal. Brains of kittens which have died have been examined, and no gross changes nor apparent histological changes could be discerned. The shaking begins to manifest itself approximately 2 weeks to a month after birth as a continual, full body, oscillating tremble which produces a pumping and rocking that is especially obvious in the rear quarters. The body and head of the cat rolls and bobs in an undulating fashion while the tail seems to revolve in circles. When at complete repose or when held very firmly, the trembling ceases, but whenever the cat is alert or moving in any manner it shakes continuously. Even when suspended by the nape of the neck, it trembles vigorously in contrast to the cerebellar hypoplasia victim which relaxes into the same quiet hanging position as an unaffected individual. In walking, the legs, although quite shaky, are placed in a more normal position and there is less stumbling sideways than in the cases of cerebellar hypoplasia. On rough surfaces, the cats tend to tumble when moving and, sometimes, while standing, but on smooth floors they are more surefooted although much slower than their normal litter mates. The severity of the effect has been much more uniform than in the hypoplasia ataxias which range from slightly to profoundly handicapped. The one affected kitten tested for swimming ability swam shakily but otherwise upright and well. A preliminary electroencephalographic study of one kitten showed significant seizure-like activity, and more extensive studies are in progress on others.

After the onset of the trembling, affected kittens begin to lag behind their siblings in development, their growth rate slows, they gain weight slowly, and only one has survived beyond 3.5 months, being now 6 months old. One died of infection resulting from a perforated intestine, the rest of those that died succumbed to respiratory infections. Currently, three living younger kittens are being studied.

The data show that matings between carriers have produced eleven affected out of a total of forty-six offspring, a very close fit to the 3/4 to 1/4 ratio expected from a single autosomal recessive factor. The ratio of sexes was six female to five male among the tremblers and eighteen to seventeen among the normals. The lack of affected kittens in any litters but those involving the original tom or his descendants, the relatively close fit to expected ratios for a genetic factor, and the marked differences in manifestation of symptoms and in brain structure from the viral caused ataxia give strong support to a conclusion that this is an hereditary syndrome of the domestic cat.

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ϵ -N-Dimethyllysine in Amoeba Actin

AMOEBA actin, a protein that shares many of the chemical, physical, and ultrastructural properties of muscle actin, has recently been isolated from *Acanthamoeba castellanii*^{1,2}. The final steps in the purification procedure are the sedimentation of polymerized amoeba F-actin, its depolymerization by prolonged dialysis against a solution of ATP, and separation by molecular filtration on 'Sephadex G-200' of a minor excluded fraction and a major included fraction (amoeba G-actin). The product is monodisperse as judged by sedimentation equilibrium centrifugation in 5 M guanidine¹ and has a molecular weight of about 39,500. Polyacrylamide gel electrophoresis at pH 9.5 in 8.5 M urea of the reduced, carboxymethylated protein shows one major band that accounts for more than 90 per cent of the stainable protein. The amino-acid composition of amoeba actin was found to be very similar to that of other actins including approximately 1 mole of the rare amino-acid 3-methylhistidine (3-MH) per mole of protein¹. In addition, an unidentified component was detected which had the same elution time as ϵ -N-methyllysine in the chromatographic system used¹ which does not separate monomethyllysine from dimethyllysine.

More detailed analyses have now demonstrated that highly purified amoeba actin contains one equivalent of ϵ -N-dimethyllysine (DML), ϵ -N-Monomethyllysine (MML) and traces of ϵ -N-trimethyllysine (TML) may also be present. All three methyllysines have previously been identified in histones^{3,4} and TML occurs in two species of cytochrome c⁵. Rabbit myosin contains MML⁷⁻⁹ and TML⁹, and MML is present in the flagella of some *Salmonella*¹⁰. Methyllysines have not previously been reported in actin from any source.

The methyllysine content of purified amoeba actin was established as follows. The "Sephadex-included" fraction (amoeba G-actin) was divided into leading and trailing halves which were separately subjected to amino-acid analyses. The amino-acid compositions of the two fractions were essentially identical which provides additional evidence for the purity of amoeba actin. The basic amino-acids were chromatographed on ion-exchange systems B and C of Kuehl and Adelstein⁹. In system B, lysine, MML, DML, TML, and histidine are well separated, but 3-MH and NH₃ tend to overlap. In system C, lysine, MML, histidine, 3-MH, and NH₃ are well separated, but DML and TML are co-eluted (Table 1). Amoeba actin contained about 2.1 mole of DML for 10⁵ g of recovered amino-acids (equal to its content of 3-MH) and about 20 per cent as much MML. The trace amount of TML was too small to quantitate. The "Sephadex-excluded" fraction contained about half the concentration of DML and 3-MH, much more TML (about 1 mole for 83,000 g of amino-acids), and much less MML. Similar results have been obtained with three other preparations of amoeba actin. Another sample of Sephadex-purified amoeba G-actin was polymerized in 0.1 M KCl and the amoeba F-actin was collected by centrifugation at 249,000g (bottom of tube) for 3 h. The pellet (F-actin) accounted for 40 per cent of the protein and the supernatant (unpolymerized G-actin) for 60 per cent of the protein. Both fractions contained 2.5 mole of DML for 10⁵ g of amino-acids (about equal to their content of 3-MH) and 10-20 per cent as much MML (Table 1). The failure of much of the amoeba G-actin to polymerize may have arisen from denaturation during the relatively long period (6-7 days) of purification that follows the polymerization step in the isolation.

Table 1. METHYLATED AMINO-ACIDS (MOLES/100,000 g) OF PURIFIED AMOEBA ACTIN

Amino-acid	Experiment I		Sephadex-excluded fraction	Experiment II	
	Sephadex-included fraction (G-actin) Leading half	Trailing half		KCl polymerization Pellet	Supernatant
MML	0.50	0.45	< 0.26	0.25	0.53
DML	2.04	2.2	1.2	2.5	2.46
TML	Trace	Trace	1.2	—	—
3-MH	1.9	1.4	0.93	2.1	2.2

In experiment I, 19 mg of protein in 6 ml. of 0.5 mM ATP-3 mM cysteine (pH 8.1), was applied to a column containing 'Sephadex G-200' (2.5 cm x 70 cm) and eluted with the same solvent. The excluded fraction (eluted between 119 and 191 ml.) contained 3.0 mg of protein and the included fraction (eluted between 196 and 265 ml.) 15.0 mg of protein¹¹. The excluded fraction and the leading and trailing halves of the symmetrical included fraction were separately hydrolysed in 6 M HCl for 72 h at 110° C. The methyllysines were separated in system B of Kuehl and Adelstein⁹ with a buffer flow rate of 45 ml./h rather than 68 ml./h. Elution times were increased by a factor of 1.5. Because ammonia and 3-MH were incompletely resolved, 3-MH was determined with system C of Kuehl and Adelstein⁹ but with the length of the column decreased from 55 cm to 18 cm, and the buffer flow rate decreased from 68 ml./h to 45 ml./h. Elution times were reduced by a factor of 2. Neutral and acidic amino-acids and arginine were independently determined by the accelerated system of Spackman¹². Standard MML and DML were purchased from Cyclo Chemical Corporation. Standard TML was synthesized by reacting methyl iodide with α -N-acetyllysine¹³.

In experiment II, 5.5 mg of purified amoeba G-actin in 3 ml. of 0.5 mM ATP-3 mM cysteine-0.1 M KCl (pH 8.1) was centrifuged for 3 h at 50,000 r.p.m. in the Spinco 'SW-65' rotor. The pellet (F-actin) contained 2.2 mg of protein and the supernatant contained 3.3 mg of protein. The basic amino-acids were analysed with system C of Kuehl and Adelstein⁹ modified as described for experiment I. This system does not separate TML from DML.

As an independent identification of the methyllysine, amoebae were grown in rotating culture¹⁴ in the presence of 10 mCi of ³H-lysine. Actin was isolated as previously described¹ through the preparation of the acetone powder of the complex between amoeba actin and muscle myosin. At this stage the radioactive material was mixed with ten times its weight of non-radioactive acetone powder. The purification was then continued to obtain purified amoeba G-actin of specific radioactivity similar to that of the mixed proteins in the crude amoeba extract when corrected for dilution by the non-radioactive carrier protein (Table 2). The actin was then hydrolysed and the specific radioactivities of the individual amino-acids were

determined (Table 2). Lysine, MML and DML accounted for 90–95 per cent of the total radioactivity. No radioactivity was present in histidine 3-MH, or ammonia. Only 5–10 per cent of the total radioactivity was recovered in the neutral and acidic amino-acids. The high specific radioactivities of MML and DML support their identifications and establish their synthesis by the amoebae. Some of the differences in specific radioactivities of the three amino-acids are undoubtedly attributable to unavoidable errors in the measurements of small amounts of amino-acids and of low levels of radioactivity in a highly quenched system.

Table 2. RADIOACTIVE AMINO-ACIDS IN ACTIN FROM AMOEBAE GROWN IN THE PRESENCE OF ³H-LYSINE

	Protein (c.p.m./mg)	Lysine	MML (c.p.m./μmole)	DML	TML
Crude amoeba extract	79,000	120,000	130,000	110,000	96,000
Purified G-actin	53,000	83,000	112,000	67,200	—

Crude amoeba extract and purified G-actin from amoebae grown in the presence of 10 mCi of ³H-lysine were hydrolysed in 6 M HCl for 72 h at 110°C. The basic amino-acids were separated by system B of Kuehl and Adelstein⁸ and the effluent from the photometer of the Beckman '120-B' amino-acid analyser was collected in fractions of 3–3 ml. Aliquots of 1 ml. were added to 15 ml. of a solution of 10 per cent naphthalene and 0.5 per cent diphenyl-oxazole in dioxane. The precipitates that formed were allowed to settle and the supernatant solutions were decanted. The precipitates were dissolved by heating for 1–2 h at 60°C in a solution of 4 per cent naphthalene, 0.2 per cent diphenyl-oxazole, and 20 per cent water in dioxane to which had been added 10–20 μl. of concentrated HCl. The samples were analysed for radioactivity in a Beckman model 'LS-250' scintillation counter and were corrected for quenching by use of the external standard. The specific radioactivities of the crude amoeba extract have been adjusted to compensate for the addition of non-radioactive carrier protein (see text). Essentially, no counts were detected in the fraction of the actin hydrolysate with the elution time of TML.

Finally, one should consider whether the methyllysines of amoeba actin might be derived from a contaminating protein even though the isolation procedure¹ is based on the unique properties of actin and the final product seems to be essentially homogeneous by sensitive physico-chemical and biochemical criteria^{1,2}. Histones (but not from all species) are the only other proteins known to contain DML and these proteins also have a very high content of lysine, histidine and arginine^{4,14}. Were the DML of amoeba actin derived from a contaminating histone with the highest content of DML yet reported^{4,5,14}, the contaminating protein would also account for between 50 and 200 per cent of the lysine, histidine and arginine. This seems highly unlikely especially because the compositions of the basic amino-acids of amoeba actin and muscle actin are nearly identical¹. Moreover, MML, DML and TML occur in histone⁵ in the ratio of 1 : 2 : 1 which is very different from that observed for amoeba actin. We conclude that amoeba actin contains 1 mole of DML and 1 mole of 3-MH per mole of protein of molecular weight about 42,000. The data for MML and TML are not conclusive. TML is present in greater concentration in the "Sephadex-excluded" proteins and its presence in purified amoeba actin might represent a contaminating protein. But if our estimate of at least 90 per cent purity for the amoeba actin is correct, such a contaminating protein would have to contain a very high percentage of methyllysines. On the other hand, amoeba actin may be molecularly heterogeneous, with certain lysine residues in various stages of methylation. These questions, and the mechanism of methylation, will only be resolved by further experimentation.

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Phthalic Acid in the Deep Sea Jellyfish *Atolla*

DURING an analysis of the lipids of the deep sea jellyfish *Atolla*, an unusually large percentage of phthalic acid was found in the fatty acids of the saponifiable fraction. The identification was based on gas liquid chromatographic (GLC) analysis on three columns (Table 1).

Table 1. GLC OF PHTHALIC ACID FOUND IN *Atolla*

	<i>t_R</i> of Standard Me 1,2 phthalate	<i>t_R</i> of unknown
5 per cent/9/NPGS at 175°C	0.424	0.430
8 per cent/5/Apiexon L at 200°C	0.077	0.077
20 per cent/5/DEGS at 175°C	1.40	1.37

Retention times are relative to 18:1 standard oleic acid.

GLC analysis was carried out on the total Me esters, hydrogenated Me esters and on the non-urea complexed esters¹. Mass spectrometry confirmed the identification of phthalic acid and indicated the 1,2 acid as the principal isomer present².

The specimens of *Atolla* and other zooplankton were taken from deep net hauls (1,000–0 m) in the North Atlantic (33° N 14° W) for lipid analysis. All material was kept in specially cleaned glass jars under nitrogen at –25°C until analysed in the laboratory. Analyses of the other animals from the deep hauls revealed no significant amounts of phthalic acid, and so contamination during the netting and storage of *Atolla* seems unlikely.

No plastic came into contact with the extract during the preparative work-up and analysis of the *Atolla* lipids, which was one of a series of similar analyses. The same glassware was used as in the other analyses, which revealed no contamination by phthalic acid. All solvents were redistilled analar reagents. Thus there is unlikely to have been any contamination during the analysis.

I have concluded that the phthalic acid was present in the animal before capture. Although it is present as only 0.01 per cent of the total animal wet weight this represents 13 per cent of the total lipid weight and 26 per cent of the total fatty acid weight. It is most unlikely that phthalic acid is present as a normal metabolite and so it seems to have been assimilated and stored by the animal.

Phthalic acid is in fairly widespread use, chiefly as a plasticizer in the plastics industry, but the occurrence of such large amounts in a deep living oceanic animal, such as *Atolla*, seems remarkable.

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When is the Male Plastome eliminated?

PLASTOME mutations result in chloroplast defects which are inherited in a non-Mendelian manner. Because normal and defective chloroplasts can exist in the same cell and be sorted out by somatic segregation, the plastome is considered to be in the chloroplasts themselves, possibly in their DNA. A strictly maternally inherited plastid mutant in *Nicotiana tabacum* (status albomaculatus) falls into this category¹. The white tissues of the variegated plants contain only structurally altered chloroplasts and mitochondria, while cells with both normal and defective chloroplasts have been observed in white-green mottled tissues. Crosses among white, green and variegated inflorescences have shown that this mutation is inherited only through the egg cell and that the male plastome is lost sometime before the first division of the zygote. To investigate whether the plastome factors of the pollen mother cell can be transferred into the pollen grains and remain functional, we have grown polyhaploid plants of this plastome mutant from anthers²⁻⁴.

Table 1. ORIGIN AND PHENOTYPE OF HAPLOID PLANTLETS ARISING FROM ANTHER CULTURE OF *N. tabacum* VARIETY SAMSUM AND A MATERNALLY INHERITED PLASTOME MUTANT

Plant phenotype	Shoot phenotype, per cent white	No. of shoots used	No. of anthers producing plantlets	No. of haploid plantlets*		
				White	Green	Variegated
Green	0 (green)	6	11	0	20	0
Variegated	0 (green)	1	1	0	1	0
"	30	1	2	10	0	0
"	30-50	2	2	0	3	0
"	50	1	1	0	0	1
"	50-60	1	1	2	0	0
"	50-80	1	12	10	2	0
"	60-95	6	1	20	0	0
"	70-95	2	1	0	0	2
"	100	6	3	3	0	3
"			23	92	0	0
"			3	6	0	0
"			2	0	6	0
"			15	54	0	0

* Numbers greater than 9 represent minimum values since exact counts were made only when less than ten plantlets grew out from an anther.

Buds were selected to contain uninucleate pollen grains⁴ from six wild type plants, variety Samsun, and nineteen variegated plants. The latter were pruned to give shoots with inflorescences visually 100 per cent white, between 30 and 95 per cent white, or 100 per cent green. We took fifty flower buds from the Samsun plants and six buds from the green shoots of the variegated plants, that is, 280 anthers from green shoots. We tried 675 anthers of 135 buds from white and variegated shoots of the mutant. Anthers were sterilized in 70 per cent ethanol, followed by immersion in 0.5 per cent sodium hypochlorite, and three washes in sterilized, distilled water. Approximately 8 per cent of cultured anthers gave rise to haploid plantlets. Although this is much lower than the 45 per cent reported by Nitsch and Nitsch⁴ for the variety White Burley, it is similar to the 12 per cent obtained by Carlson⁵ and the 6 per cent of Nakata and Tanaka³. Pollen grains in anthers from white and variegated shoots developed into haploid plantlets as well, if not better, than those taken from green shoots (Table 1).

The aseptically removed anthers were cultured on medium H (ref. 4), containing salts, organic substrates and 0.1 mg/l. of IAA. After growing to 4-6 cm, green plantlets were potted in peat-vermiculite. Variegated and white haploids were subcultured on medium T—medium H lacking IAA⁴—before being potted. The more white tissue present in the haploids, the slower they grew, and the longer they had to be cultured on medium T (Fig. 1). Cultures and haploid plantlets were kept in a 'Percival PGW8' growth chamber with a 16 h day (15,000 lux) at 28° C and an 8 h night at 22° C. Ploidy level of

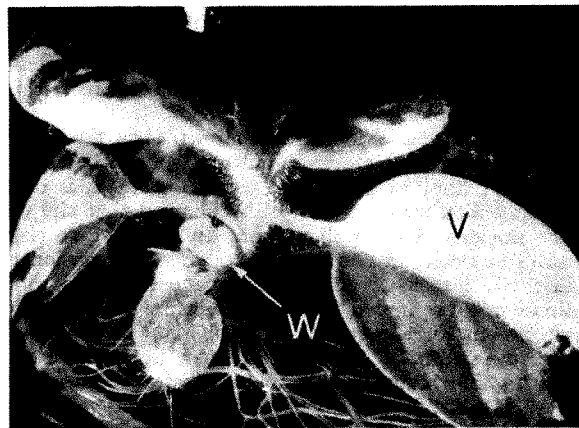


Fig. 1. After 88 days of culture both a white (w) and a variegated (v) haploid plantlet can be seen emerging from a single anther of a variegated shoot having 60-95 per cent white tissue ($\times 1.7$).

the plantlets was determined by chromosome counts of root tip cells and/or ability to set viable seed.

All plantlets obtained from anthers on green shoots and some of those emerging from variegated shoots were green (Table 1). Seven which were sterile and had twenty-four chromosomes were classified as haploids, so were another thirty green plants which were unable to set seeds. Two Samsun anthers gave, in addition to haploids, one diploid plant each, as chromosome counts and fertility showed (compare ref. 6).

Anthers from entirely white shoots gave rise to white plantlets only (Table 1). If kept under low light intensity (600 lux) on medium T, these white plantlets continued to grow for more than 6 months; after which they had eighteen visible leaves and were approximately 4 cm tall. Their growing points had a faint green colour, as described for the growing points of white shoots from diploid variegated plants¹. Although the slow growth of the white plantlets made chromosome counts difficult, examination of root tips emerging from three of the eight anthers studied yielded counts of approximately twenty-four, the haploid number of the allopolyploid, *N. tabacum*. The production of white haploids from white anthers demonstrates that the mutated plastome factor is retained unaltered in the uninucleate pollen grains, just as in the egg cells.

Green, white and variegated haploid plantlets were obtained from the anthers of the variegated shoots (Table 1). More than one phenotype can arise in a single anther: for example, one anther from the shoots classified as 50-60 per cent white produced ten white and two green haploid plantlets, whereas an anther from the 60-95 per cent white shoots yielded three white and three variegated haploid plantlets (Fig. 1). These phenotypic differences within anthers reveal that the segregation of the two plastome factors continues at least until the divisions of the sporogenous tissue from which the pollen mother cells differentiate, and possibly even until the second meiotic division. Correspondingly, the developmental stage at which segregation of the plastome factors occurs determines whether the anthers of an individual bud produce haploid plantlets of one or more phenotypes. That most of the variegated shoots in Table 1 gave rise to haploid plantlets of only one phenotype is probably due to the low percentage of plantlet-producing anthers obtained in these experiments.

Of the six variegated plantlets obtained (Table 1) two were grown to an adult stage and found to be haploids, both by chromosome counts and sterility. The other four plantlets did not survive transplantation from medium T to the peat-vermiculite mixture because they had too little green tissue. The occurrence of these six

plants shows that at least two different plastome factors must have been present and remained functional in the uninucleate pollen grains, just as in the egg cells. Furthermore, these two factors segregate somatically from mixed cells in the haploids as in the diploids.

Sunderland and Wicks⁷ have shown that the optimal and last stage for starting cultures of anthers of *N. tabacum*, if haploid plants are to be obtained, is during the first pollen mitosis. They also showed that the vegetative nucleus of the pollen grain divides to give rise to most of the cells in the haploids. This information pinpoints the latest developmental stage at which pollen grains can be removed from their normal to an artificial environment for the growth of haploids. In conjunction with our results, it follows that even in cases of strict maternal inheritance, the male plastome factors are present in a stable and functional form in the pollen grain on the plant at the time of first mitotic division. At what subsequent stage of pollen maturation, pollen tube growth, fertilization or first zygote division the paternal plastome is eliminated will have to be determined by other methods.

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Plant Cells killed by Soft Rot Parasites

A RECENT report¹ has focused attention on the use of cell wall degrading enzymes to isolate protoplasts from plant cells. The aim is presumably to obtain viable protoplasts with metabolic activities close to those of protoplasts *in situ*. For many years, however, plant pathologists have been concerned with why in soft rots and other plant diseases characterized by cell separation protoplasts are rapidly killed. There is an apparent contradiction here.

Brown² established the close connexion between the action of thermolabile factors that cause cells to separate along the line of the middle lamella and death of plant protoplasts. (This and later related work was reviewed by him in 1965³.) More recently it has been established with reasonable certainty that cell separation characteristic of soft rots is caused by enzymes which rupture glycosidic linkages between adjacent galacturonic residues that dominate polymers of pectic substances in the middle lamella and matrix of the primary cell wall. The mechanisms by which these enzymes act *in vivo* is still not known. The enzymes are polygalacturonases that rupture linkages by hydrolysis or lyases that act by *trans*-elimination⁴⁻⁶.

Since 1968 we have been studying soft rots of potato tubers caused by a bacterium, *Erwinia carotovora* var. *atroseptica*, and a fungus, *Corticium praticola*. We have

established that the enzyme causing cell separation in *C. praticola* rots is a polygalacturonase, pH optimum 4.0-5.0, inhibited by Ca^{2+} , and, in contrast, that the corresponding enzyme in *E. carotovora* var. *atroseptica* rots is a pectate *trans*-eliminase, pH optimum 9.0-10.0, activated by Ca^{2+} . There is a close connexion between cell separation caused by these two enzymes and death of protoplasts as assessed by absence of plasmolysis in hypertonic solutions.

Extracts from rots also have striking effects on the permeability of protoplasts long before they separate cells in tissue disks. Thus in an extract which separates cells in about 60 min a readily detectable leakage of electrolytes from cells occurs in 10 min; this increases steadily to a maximum before the tissue loses coherence. This marked increase in permeability to electrolytes is closely related to the activity of the polygalacturonase or the *trans*-eliminase as affected by heat, pH and Ca^{2+} , and to the activity of these enzymes in fractions separated by precipitation with $(\text{NH}_4)_2\text{SO}_4$. This and other evidence strongly suggests that the polygalacturonase or *trans*-eliminase of these pathogens which causes cell separation also alters cells rapidly so that their permeability to electrolytes is greatly increased, and that these changes culminate in the death of the protoplasts at about the time when the tissue has lost its coherence.

Extracts containing cell separating enzymes also cause cells to lose water rapidly. Thus disks of potato tubers lose about 25 per cent fresh weight in about 40 min when placed in extracts which cause cell separation in about the same time (personal communication from L. E. Webb).

It has been suggested⁷ that the lethal effects of extracts from rotted tissue or from filtrates from cultures of soft rot pathogens depend on the phosphatidases they contain. But fractional precipitation of *E. carotovora* var. *atroseptica* preparations with $(\text{NH}_4)_2\text{SO}_4$, clearly separates phosphatidases and enzymes causing cell separation and increases in permeability both of which are maximal in the same fractions. For *C. praticola* preparations the separation is not so distinct; some fractions shows all three activities; but others that separate cells and increase permeability have no, or only very low phosphatidase activity.

This evidence, though indirect, again confirms Brown's idea that the enzymes causing cell separation also kill protoplasts. The question, now as in the past, is how they do so. There is also the question of the use of cell wall degrading enzymes to obtain apparently viable protoplasts. The success of this technique probably depends on the fact that the enzymes are used in a plasmolyticum that prevents protoplasts from bursting. Tribe⁸ made the significant discovery that the killing normally associated with cell separation is greatly retarded when protoplasts are plasmolysed. We find that plasmolysis decreases markedly the leakage of electrolytes that would otherwise occur in extracts from rots.

It therefore seems that the killing of cells by cell separating enzymes may be primarily a physical effect. Normally the protoplast is constrained within a cellulose microfibrillar envelope embedded in the matrix of the cell wall which is continuous with the middle lamella. Matrix and middle lamella are probably highly hydrated, very firm gels.

At the time when cells are free to separate through the action of polygalacturonase or *trans*-eliminase, and probably for some time afterwards, most of the matrix of the primary wall and all that of the secondary wall is probably intact; so is the microfibrillar meshwork. This implies that the plasmalemma within the lumen of the cell remains separated from the external solution by almost all the original cell wall. But progressive dissolution of the middle lamella, and possibly the inner layers of the primary wall, will presumably expose the plasmalemma to the ambient solution at the plasmodesmata. This may account for the rapid increase in permeability that occurs before the middle lamella is sufficiently degraded to allow

cell separation. And somewhat later the plasmalemma could be ruptured by osmotic forces acting at plasmodesmata when cells are in hypotonic solutions. It is not easy to see where else the plasmalemma would be exposed by the rapid action of cell separating enzymes. A study of the middle lamella and cell wall and the behaviour of the plasmalemma of and near plasmodesmata in cells exposed to cell separating enzymes may well explain how they are killed by soft rot parasites.

While this article was in preparation an abstract⁹ has been published giving similar results for *Erwinia carotovora*.

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Therapeutic Effects of Fluorene, Phenanthrene and Xanthene Derivatives on Fungal Diseases of Tomato

DERIVATIVES of 9-hydroxyfluorene-9-carboxylic acid, known as Morphactins, have therapeutic effects on several diseases of tomato¹. Apparently, there is no correlation between the morphoregulatory and therapeutic activities of these compounds. Davis *et al.*² have shown that unsubstituted fluorene also has a certain effect on *Fusarium* wilt of tomato. This prompted us to examine several derivatives of fluorene for their therapeutic potencies against two vascular diseases (caused by *Fusarium oxysporum* f. *lycopersici* and *Verticillium albo-atrum*) and one leaf disease (caused by *Phytophthora infestans*).

Tomato plants, variety Bonny Best, grown in sand culture in the greenhouse, were inoculated at the seven to eight leaf stage with *Fusarium* by dipping the roots into a suspension of mycelium and conidia. The compounds to be tested were watered on to sand cultures with the plants *in situ*, before and/or after inoculation. All experiments were carried out with six replicates. All substances were also tested several times in different concentrations and applied at different times. About 3 weeks after inoculation wilt symptoms on the leaves and vascular browning in the stems were evaluated. Individual leaves were examined and classified according to the degree of wilting or yellowing on a scale of 0 to 4. The "index of wilt symptoms" (*I*) was obtained from

$$\frac{\text{Sum of leaf ratings}}{\text{Number of leaves}} \times 100$$

To obtain the "index of vascular browning" (*I'*) stems were cut within each internode and the browning of the three main vascular bundles was rated on a scale from 0 to 3. The index was calculated from

$$\frac{\text{Sum of vascular bundle ratings}}{\text{Number of cut internodes}} \times 100$$

Table 1 shows the result of an experiment in which several fluorene derivatives were applied 2, 5, 8, 11 and

14 days after inoculation. All the compounds investigated drastically reduced the disease symptoms caused by *Fusarium*; the differences are highly significant compared with the untreated control. Vascular browning was usually reduced less than the symptoms of wilt. When the compounds were applied before infection the effect was about the same; most effective, however, were combined treatments, given both before and after inoculation. The effect of application to the leaf, on the other hand, was limited.

Table 1. INFLUENCE OF SOME FLUORENE DERIVATIVES (10^{-4} M) ON *Fusarium* WILT OF TOMATO WHEN APPLIED AFTER INFECTION

Treatment	Index of wilt symptoms	Index of vascular browning
Untreated control	78.2	93.5
2-Bromofluorene	8.4	13.6
9-Bromofluorene	22.3	26.5
9-Chlorofluorene	17.6	26.7
9-Hydroxyfluorene	12.0	22.7
2-Acetylfluorene	9.7	34.1
2-Nitrofluorenone	11.4	21.2

In further experiments the development of the fungus in the stems was evaluated according to Saaltink's method³. The stem of each plant was divided into equal sections which were disinfected on the outside and homogenized in standardized conditions. Dilutions were prepared from these homogenates, mixed with malt agar and incubated in Petri dishes. The number of developing *Fusarium* colonies, based on 1 g of stem tissue, served as a measure of the amount of fungus within the stem. They showed that the development of *Fusarium* mycelium in the vascular system of infected plants is strongly restricted by the compounds applied. There is, however, no correlation between the therapeutic effect of the compounds *in vivo* and their fungitoxicity *in vitro*, which was determined by the growth of mycelium on Czapek-Dox-Agar and by spore germination tests, using concentrations of between 10^{-3} and 10^{-5} M at 27°C. Some of the most effective therapeutic substances, such as 2-acetylfluorene and 2-nitrofluorenone, proved to be only weakly fungitoxic *in vitro*. Apparently they counteract the disease in a more indirect way.

Because of their structural similarity to the fluorenes a series of phenanthrene derivatives was also tested. Some had a strong therapeutic effect against *Fusarium* wilt when applied through the roots before and/or after infection (Table 2).

Table 2. INFLUENCE OF SEVERAL PHENANTHRENE DERIVATIVES (10^{-4} M) ON *Fusarium* WILT OF TOMATO

Treatment	Index of wilt symptoms	Index of vascular browning
Untreated control	88.2	95.3
Phenanthrene	43.5	73.1
2-Acetylphenanthrene	46.2	78.4
3-Acetylphenanthrene	6.3	42.7
1-Methylphenanthrene	5.9	31.5
1,2,3,4,5,6,7,8-Octahydrophenanthrene	0.3	7.6

Compounds were applied 2, 4 and 6 days after inoculation.

The same is true for the structurally related xanthene compounds, of which xanthene-9-carboxylic acid, thioxanthene, 2-chlorothioxanthene, thioxanthone and 2-chlorothioxanthone had a preventive as well as a curative effect against *Fusarium* wilt, although *in vitro* they were only slightly toxic to the pathogen.

Similar results were also obtained with fluorene and phenanthrene derivatives against *Verticillium* wilt of tomato. In this case the preinfection application through the roots proved superior to postinfection application; but the best effect again was found when treatment was given before as well as after inoculation (Fig. 1). Xanthene compounds were not tested against *Verticillium*.

Phytophthora infestans was sprayed onto the leaves of the tomato plants at the six to eight leaf stage in a suspension of sporangia (about 40,000 sporangia/ml.).



Fig. 1. Influence of 2-acetylfluorene (10^{-4} M), applied before as well as after inoculation, on *Verticillium* wilt of tomato. From the left: infected/untreated; infected/treated; noninfected/treated; noninfected/untreated.

Plants were then kept in a humid chamber until leaf symptoms were evaluated about 5–6 days after infection. All substances tested were applied before inoculation. Some fluorene derivatives proved highly effective, especially when applied to the leaves. But a few compounds had a strong effect after application to the roots too—clearly they acted systemically (Table 3). When applied to the leaves, 9-aminofluorene hydrochlorid was more effective than the other compounds; it prevented infection when applied in a concentration of 5×10^{-4} M. Other compounds, however, which were highly effective against the vascular diseases (for example, 2-acetylfluorene), did not affect *Phytophthora* to the same degree.

Table 3. INFLUENCE OF SOME FLUORENE DERIVATIVES (10^{-4} M) ON THE INCIDENCE OF *Phytophthora infestans* ON TOMATO PLANTS (RELATIVE VALUES)

Treatment	Leaf application*	Root application†
Untreated control	100.0	100.0
Fluorene	55.3	39.0
2-Bromofluorene	10.4	22.0
9-Bromofluorene	48.9	68.8
9-Chlorofluorene	20.1	57.5
9-Hydroxyfluorene	17.7	44.8
2-Acetylfluorene	34.1	73.9
2-Nitrofluorene	29.7	18.6
9-Aminofluorene-hydrochlorid	2.6	42.0

* 5, 3 and 1 days before inoculation.

† 6, 4 and 2 days before inoculation.

Several phenanthrene compounds were ineffective against *Phytophthora* even when applied to the leaves. Xanthene derivatives have not been tested so far.

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Designation of Physiological Races of Plant Pathogens

THE physiological races of most plant pathogens have been designated on an historical basis, being numbered in order of their isolation and identification. This has little intrinsic merit, because the order in which they are isolated depends largely on their relative frequencies within the population, and these frequencies vary with

changes in the host population¹. Moreover, rare races are likely to escape detection altogether, so that the total of known races is not a reliable guide to the inherent variability of the pathogen.

In addition, the designated code number of a race bears no relation to its spectrum of pathogenicity on the set of differential hosts. Thus a key to the known races is essential when identifying new isolates of the pathogen, and in the interpretation of other reports involving physiologic specialization. Because the number of known races of certain pathogens runs into hundreds², consulting a key can be extremely laborious.

A system generally accepted to be ideal is that in current use for *Phytophthora infestans* (Mont.) de Bary, in which a physiological race is designated by the virulence genes it carries or by the resistance genes it can overcome³. Even this system can be criticized, on the grounds that the nomenclature of complex races, such as race (1, 2, 3, 4, 5, 6, 7, 8, 9), can be cumbersome. More important, however, is the fact that it cannot be applied to the many host-pathogen relationships in which the specific genes have not been investigated, and it is complicated in those for which the "gene for gene" hypothesis⁴ may not hold. For these pathogens, the differential host, rather than the resistance or virulence gene, remains the basis of race identification.

I propose a new system of nomenclature, in which the designation of a race is derived from the spectrum of pathogenicity against either differential varieties or resistance genes. It can thus be used whether or not the genetic basis of resistance in the host has been elucidated.

Ideally, the system requires that the reactions of the differential hosts to each isolate can be clearly divided into two categories, resistant or susceptible. But if the use of intermediate categories is absolutely essential (as when a host exhibits resistant, intermediate and susceptible reactions to different isolates, and these cannot be reduced to two categories by the elimination of modifying genes or the manipulation of environmental conditions), then the scale can be expanded to three, four or more categories, although the system then becomes more complex.

To designate a race, the differential hosts are first arranged in a fixed linear order. (The rigidity of this order is imperative but presents no great difficulty.) The reaction of each host to a given isolate is then written down, using 0 for resistance and 1 for susceptibility. The resulting series of 0s and 1s is then considered as a binary number and converted to decenary notation, giving a simple, unique number for each potential race. The mechanics of this process are best illustrated by the following example, in which an isolate is pathogenic on three of the seven differential hosts, namely E, C, and B:

Differential host	G	F	E	D	C	B	A
Race reaction	R	R	S	R	S	S	R
Binary value	0	0	1	0	1	1	0
Decenary value	(2 ⁶)	(2 ⁵)	2 ⁴	(2 ³)	2 ²	2 ¹	(2 ⁰)

Designation of isolate = $16 + 4 + 2$ = race 22.

Similarly, the spectrum of pathogenicity of a given race can be simply obtained from its designation. Thus race 85 (= $64 + 16 + 4 + 1$ = $2^6 + 2^4 + 2^2 + 2^0$ = 1010101) attacks hosts G, E, C and A only.

Often, differential hosts supplementary to the original series are later required. These may be added to the left end of the series, so that "old" races which do not attack these hosts retain the same number, while those that do acquire a new number. The order in which the differentials are arranged can also facilitate the interpretation of a race designation, since it is clear in the above example that only odd numbered races are pathogenic on differential A and only those numbered 64 or higher are pathogenic on G.

This system could, with great advantage, be adopted for the description of physiological variation in a wide

range of disease organisms, where clear differences in host reactions to different isolates have been demonstrated.

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Key Generation by Computer

KEYS for the identification of specimens have been used by biologists for several centuries. A method is described here for generating such keys automatically by computer, instead of composing them by hand, which until recently was the only way to produce them. The use of keys is not confined to biology, and applications for the computer method have been found in the earth sciences, medicine and engineering. Fig. 1 is an example of a computer-produced key for the British species of plants of the genus *Epilobium* L. (Willow-herb). It should be emphasized that the key-generating program is primarily a tool for the identification of objects and not for their classification, although it is in fact capable of creating a crude classification for the purposes of constructing a key.

Keys of the traditional kind are often not available for specialists or students in the less popular branches of biology. There are shortcomings in such keys, even when they are available: (a) the key may not entirely agree with the corresponding description, because of the complexity of the task of key building; (b) the key is unlikely to be optimized to give the shortest average path to an identification; and (c) there is no easy way to allow for adding taxa, altering taxon descriptions or expressing preferences in the choice and ordering of the characters used.

Computer-generated keys, on the other hand, are as accurate as the data they are built with, and can be optimized to reduce the number of characters used and length of key produced. The optimization can also take account of the fact that characters of taxa are not all equally useful or suitable by giving weights to the characters and building the key accordingly, and allow for the fact that some taxa are common while others are rare. A given key can be regenerated with little extra effort when editing is required.

The information describing the taxa (objects, species) is expressed as a rectangular matrix of characters (features, attributes) against values. For example, *Epilobium roseum* has the property that its stigma is entire. A character and value pair appropriate to this could be "stigma" and "entire" respectively. The character and value are stored in computer memory as strings of symbols. The choice of characters and values is left entirely to the user. Two or several different values can be used with each character—in other words, characters can be binary or multi-valued. A value can equally well be qualitative ("bifid") or quantitative ("2 to 5 mm"). Values can also be missed out if they are unknown or inapplicable.

Although the program in its present form prefers to produce dichotomous keys, polychotomous keys are possible, and are most likely when multi-valued characters are frequent. The user may specify the maximum number of characters that may be considered in combination at each branch of the key. The user may choose to give all the characters equal importance, or give them any sequence of weighting that may be preferred. It is by this means that keys can be tailored to suit specimens gathered only in winter, or only as preserved in museum collections, or according to other preferences. It is also possible to weight the taxa, so that the algorithm tries to give a short identification route to a commonly occurring taxon.

Either of the two common forms of key can be produced, with indentation if required. Provision is made for highly distinctive taxa to be recognized and keyed out early, but this is only allowed to occur rarely because it usually interferes with the attempt to optimize the

KEY TO EPILOBIUM SPECIES

- | | | |
|----|--|---------------------|
| 1 | LEAVES SESSILE OR SUBSESSILE. | 2 |
| 2 | STEM WITH RAISED LINES. | 3 |
| 3 | STEM UP TO 20 CM, LEAVES NOT DECURRENT, LEAVES SINUATE TOOTHED, FLOWER DIAMETER UP TO 6 MM, FRUIT STALK 2 TO 5 CM, PLANT DECUMBENT TO ASCENDING. | E. ANAGALLIDIFOLIUM |
| 3 | STEM BETWEEN 20 AND 80 CM. | 4 |
| 4 | BASE OF LEAVES CUNEATE, LEAF MORE OR LESS SHINY ABOVE, GLANDULAR HAIRS ABSENT FROM CALYX TUBE. | E. ADNATUM |
| 4 | BASE OF LEAVES ROUNDED, LEAF DULL ABOVE, GLANDULAR HAIRS PRESENT ON CALYX TUBE. | E. OBSCURUM |
| 2 | STEM MORE OR LESS TERETE. | 5 |
| 5 | STEM SUBGLABROUS OR WITH APPRESSED SIMPLE HAIRS, STIGMA ENTIRE, GLANDULAR HAIRS ABSENT FROM STEM, LEAVES SUBGLABROUS (EXCEPT PERHAPS FOR MARGINS AND VEINS), LEAVES ENTIRE OR SUBENTIRE, FLOWER DIAMETER UP TO 6 MM. | E. PALUSTRE |
| 5 | STEM WITH SPREADING SIMPLE HAIRS THROUGHOUT. | 6 |
| 6 | STEM OVER 80 CM, LEAVES SEMIAMPLEXICAUL, STIGMA LONGER THAN STAMENS, BASE OF LEAVES CUNEATE, FLOWERS ROSE, FLOWER DIAMETER OVER 10 MM. | E. HIRSUTUM |
| 6 | STEM BETWEEN 20 AND 80 CM, LEAVES NOT AMPLEXICAUL, STIGMA ABOUT EQUAL TO STAMENS, BASE OF LEAVES ROUNDED, FLOWERS PINK, FLOWER DIAMETER 6 TO 10 MM. | E. PARVIFLORUM |
| 1 | LEAVES (AT LEAST SOME) DISTINCTLY STALKED. | 7 |
| 7 | BASE OF LEAVES CUNEATE. | 8 |
| 8 | LEAVES DECURRENT, FLOWER BUDS ERECT, FLOWER DIAMETER OVER 10 MM. | E. LAMYI |
| 8 | LEAVES NOT DECURRENT. | 9 |
| 9 | STIGMA FOUR-LOBED, GLANDULAR HAIRS ABSENT FROM STEM, STIGMA SHORTER THAN STYLE, LEAVES ELLIPTICAL TO ELLIPTICAL-LANCEOLATE, FLOWER DIAMETER 6 TO 10 MM. | E. LANCEOLATUM |
| 9 | STIGMA ENTIRE, GLANDULAR HAIRS PRESENT ON STEM, STIGMA ABOUT EQUAL TO STYLE, LEAVES OVATE-TO LANCEOLATE-ELLIPTIC, FLOWER DIAMETER UP TO 6 MM. | E. ROSEUM |
| 7 | BASE OF LEAVES ROUNDED. | 10 |
| 10 | FLOWER DIAMETER UP TO 6 MM. | 11 |
| 11 | GLANDULAR HAIRS PRESENT ON STEM, LEAVES OBLONG-LANCEOLATE, LEAVES DENTICULATE, STEM NOT ROOTING AT NODES, FLOWERS TERMINAL, PLANT ERECT, LEAVES OPPOSITE AND ALTERNATE. | E. ADENOCaulon |
| 11 | GLANDULAR HAIRS ABSENT FROM STEM, LEAVES BROAD OVATE TO SUBORBICULAR, LEAVES ENTIRE OR SUBENTIRE, STEM ROOTING AT NODES, FLOWERS AXILLARY, PLANT PROSTRATE, LEAVES ALL OPPOSITE. | E. NERTERIOIDES |
| 10 | FLOWER DIAMETER 6 TO 10 MM. | 12 |
| 12 | STEM BETWEEN 20 AND 80 CM, STIGMA FOUR-LOBED, LEAVES DENTICULATE, STEM MORE OR LESS TERETE, FRUIT STALK 0.5 TO 2 CM, PLANT ERECT, LEAVES ALL OPPOSITE. | E. MONTANUM |
| 12 | STEM UP TO 20 CM, STIGMA ENTIRE, LEAVES SINUATE TOOTHED, STEM WITH RAISED LINES, FRUIT STALK 2 TO 5 CM, PLANT DECUMBENT TO ASCENDING, LEAVES OPPOSITE AND ALTERNATE. | E. ALSINIFOLIUM |

Fig. 1. Computer-produced key for British species of plants of the genus *Epilobium* L.

key. If the taxa happen to be not all distinguishable one from another, then a partial key is formed such that the taxa may key out several at a time, instead of singly. The character matrix has to be sufficient in a certain sense for it to be possible to form a key at all. For example, at least one character has to be fully scored. It is always possible to make the matrix sufficient provided that the classification on which the taxa are based is a proper one. The user gives the names of the taxa as part of the data; all can be different, or some can be repeated. In the latter case, unnecessary key branches involving only different descriptions of a particular taxon are suppressed automatically.

Osborne¹ has shown, on the assumption that the most efficient key has the shortest average path for the identification of all taxa, that the key should be dichotomous and that, at each dichotomy, the appropriate group of taxa should be divided into two sub-groups of equal size. In practice this ideal should be approached as closely as possible.

The algorithm uses an empirically derived positive function (separation function) to choose new key branches. The separation function is a minimum for the best choice. Many functions could be used; the following has been found satisfactory. The separation function is $F = F_1 + F_2$ where $F_1 = (K - 2)^2$ and

$$F_2 = \sum_{i=1}^K \left| 1 - \frac{n_i K}{N} \right|$$

where N taxa at a node of the key are divided into K sub-groups each of size n_i , $i = 1, 2, \dots, K$. F_1 increases rapidly for divisions into more than three branches ($K \geq 3$). F_2 is zero if all the sub-groups are of equal size, (that is, $n_i = N/K$), and increases the more the n_i differ from one another. This formula for F_2 is modified for use with taxon weights. The weight w_j of the j th taxon is a positive integer such that there are effectively w_j instances of taxon j present instead of 1. Morse² and Hall³, both use a similar technique. The new effective total number of taxa is then

$$N' = \sum_{j=1}^N w_j$$

and the n_i become effectively n'_i , where n'_i is the sum of the weights of taxa in sub-group i . F_2 is then

$$\sum_{i=1}^K \left| 1 - \frac{n'_i K}{N'} \right|$$

The formula for F is derived as follows. If a character or set of characters is found to divide N taxa into K groups, then ideally $K = 2$ and $n_i = N/K$. F_1 is zero for $K = 2$, and thus favours a dichotomous key. For equal sub-division, $n_i K/N$ is unity, that is, $1 - n_i K/N$ is zero; hence F_2 is zero for equal sub-groups. The function F as used here has the advantage that it can be used to take a short cut when trying out possible key branches, for it is usually possible to find a value of F for small K which is less than the smallest possible value of F for larger K .

The key is stored in computer memory in a form of list structure based on a free storage (dynamic memory allocation) scheme. A pushdown stack is used in processing this list structure. In spite of the unsuitable nature of the language, the program is written in FORTRAN so that it can be used outside the computing centre of its origin. The computational aspects of the program are described in detail in ref. 4.

There are a number of programs concerned with various aspects of constructing keys, particularly with numerical methods for choosing key branches; they neglect, however, the substantial problems of producing a printed key, as for example the program of Hall³ and

other references in ref. 4. Hall's program shows considerable refinement in dealing with quantitative data of the kind used in numerical taxonomy, rather than qualitative data.

The only comparable program which produces a printed key is that of Morse^{2,5}. This is an independent effort, differing considerably in detail. Characters and values are combined in an indivisible couplet, and it is not yet possible to have more than one character (couplet) per lead.

On-line question and answer systems exist which assist identification in the manner of a key⁶. No printed key is produced, and such systems are rather simpler from the computing point of view.

A variety of botanical keys have been constructed. A key to eighty-six cultivated varieties of potato was produced for Mr T. Webster of the National Institute of Agricultural Botany. This was much welcomed because the task of constructing this key by hand had proved daunting. A key for 134 European umbelliferous genera was constructed from the *Flora Europaea*, vol. 2, which proved to be 20 per cent shorter than the original. A computer-generated key for sixty-four European species of *Veronica* proved to be 10 per cent shorter and used less than half the number of characters of an independent hand-made version. One may conclude that the optimizing ability of the program is more marked for higher numbers of taxa. There is evidence that the computer-generated keys are approaching the quality of the hand-made versions. Other keys have been generated for *Epilobium* (Fig. 1), *Rubus fruticosus* microspecies, *Alchemilla* and *Cortinarius*.

Keys for insects of the order Psocoptera are being generated at the University of Leeds. A key for diseases of the thyroid gland has been constructed from data on sixty-six case histories provided by Professor W. Card of the University of Glasgow, and is currently being evaluated.

Applications have been found in engineering, where the problem is not to identify a specimen but to choose a small part with desired characteristics from a catalogue. Keys for bolts used in motor cars and for transistors used in electronic circuits have been generated. A key for recognizing different types of rock from the minerals they contain has also been produced.

Applications may also be found in geology, soil science and microbiology and perhaps in psychology and archaeology.

The largest example quoted above (134 taxa) took 4 min to run in 32 K of storage (16 K of program plus 16 K of data) in the Cambridge TITAN computer, which has a cycle time of 2 μ s. Considerable economies in both space and time could be made by recoding in some other computer language if necessary.

The construction of identification keys has thus been automated. Further work is required to produce automatically keys of the same quality as is possible by hand, but this seems quite feasible. The problems connected with the labour required in collecting and verifying the data on which the key is based need attention. Computer programs can also be devised to assist in these processes. The FORTRAN key-generating program is available for general distribution.

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¹ Osborne, D. V., *New Phytol.*, **62**, 144 (1963).

² Shetler, S. G., Beaman, J. H., Hale, M. E., Morse, L. E., Crockett, J. J., and Creighton, R. A., in *Proc. Symp. Advances in Data Processing for Biology and Geology* (Academic Press, New York, in the press).

³ Hall, A. V., *Taxon*, **19**, 12 (1970).

⁴ Pankhurst, R. J., *Computer J.*, **12**, 145 (1970).

⁵ Morse, L. E., *Amer. J. Bot.*, **55**, 737 (1968).

⁶ Goodall, D. W., *Bioscience*, **18**, 485 (1968).

Book Reviews

CHROMOSOME ABNORMALITIES

Human Population Cytogenetics

Edited by Patricia A. Jacobs, W. H. Price and Pamela Law. (Pfizer Medical Monographs, No. 5.) Pp. 325. (Edinburgh University: Edinburgh, April 1970.) 75s.

CYTOGENETICS is the study of the genetical implication of chromosome behaviour. The basic principles of this discipline were laid down fifty years ago by the cytological analysis of numerical and structural changes in the chromosomes of plants. Though it was already suggested in 1930 that certain congenital aberrations in human development, for example, mongolism (Down's syndrome), may be associated with chromosome abnormalities, it was only in 1959 that the chromosomal basis of the syndrome was demonstrated by the finding of an extra chromosome in affected persons. Technical difficulties caused the delay in the study of chromosomes in man, but during the later years of the 1950s, gradual improvements in cytological methods, and particularly in the application of *in vitro* culturing of blood cells, made it possible to scrutinize, ascertain the correct number and to characterize the morphology of the human chromosomes. During the past decade extensive cytogenetical studies have been carried out on man's normal chromosomes and chromosome variants—many of which produce gross, often fatal developmental abnormalities—and they are now better known than those of any other mammals.

Cytogenetical surveys have revealed a wide spectrum of chromosome anomalies, their phenotypic consequences, and have also ascertained their frequency in different human populations. The credit for this pioneering work belongs to the Edinburgh group of investigators whose findings have stimulated many others to undertake similar surveys. This book contains the seventeen formal papers presented during the Symposium on Human Population Cytogenetics, held in Edinburgh in 1969; initiated and organized by the late Professor W. M. Court Brown, founder of the Medical Research Council's Clinical and Population Cytogenetics Research Unit in Edinburgh, and to whom the volume is dedicated.

The contributions given at this international gathering of experts summarized the findings of their surveys and showed the rapid progress which had been made in this field. R. G. Edwards (Cambridge), in his report on the maturation of oocytes and spermatocytes, described anomalies occurring at meiosis which might have a bearing on the origin of chromosome deletion and non-disjunction. The behaviour of structurally aberrant chromosomes at male meiosis was discussed and illustrated with excellent photographs by M. Hulten and J. Linsten (Stockholm). Their study of multivalent chromosome association and chiasma frequencies is the first detailed analysis of meiosis so far undertaken on human chromosomes. It was also shown that chromosome association during meiosis can disclose abnormalities which could not be identified by studies of mitotic chromosomes. Several papers dealt with the transmission and segregation of chromosome abnormalities, particularly that of translocation, the carriers of which do not show phenotypic anomaly or reduced fertility. The analysis of large numbers of such families suggests that the risk of having an affected child from a translocation carrier mother lies between 10–15 per cent: from a father the risk is about 5 per cent; it was stressed, however, that our present assessment is incomplete, because of the difficulties in detecting carriers.

Chromosome anomalies in 221 unselected spontaneous abortions were discussed by D. H. Carr (Hamilton, Canada), 36 per cent being found to have chromosome abnormality. He reported that the incidence is high when the mother's age is below 20 or above 30 years; it is also influenced by the age of the foetus, being highest in the embryos of the first trimester. His findings also indicate geographical variation in the frequency of chromosome anomalies. H. A. Lubs and F. H. Ruddle (New Haven, USA) reported that of 4,400 babies born in the same year, one in 200 had major chromosome variants (translocation and sex chromosome anomalies). These investigators have observed an association of viral epidemic with the occurrence of a cluster of the same chromosome anomaly in babies born during the same month, and have found that minor aberrations of chromosomes in D, E, and G groups were twice as frequent in black newborns as in white. The latter observation confirms a similar finding by two other research teams and suggests that racial polymorphism does occur, and it may be necessary to undertake further studies to define the race specific norms of human chromosomes. The incidence of chromosome anomalies observed in different groups of human populations were summarized by P. G. Smith and Patricia A. Jacobs (Edinburgh). The nature of the various environmental factors, and the kind of changes they produce in chromosomes, were discussed by H. J. Evans (Aberdeen). The implication of chromosome aberrations on the genetic structure of our species and the role of selection constituted the contribution by J. H. Edwards (Birmingham).

The cytogenetical studies of the past decade have brought to light facts, some vitally important for the human race, which should be applied in medical-social practice. But if progress is to be made in this direction more information will be required which can only be obtained by surveying larger groups of the human population. This will become possible by introducing automated techniques for data collecting and processing. Three contributions in this book deal with this need, discussing the various systems which could supplant the tedious, time-consuming manual steps in cytogenetic analysis and the problems to be solved for constructing such systems.

The book is an important source of information concerning the chromosomal basis of congenital anomalies and mental aberrations in man, and should interest geneticists, paediatricians, clinicians, administrators and social workers.

P. C. KOLLER

MEMBRANE STUDY

Introduction to the Study of Biological Membranes

By M. Cereijido and C. A. Rotunno. Pp. x + 261. (Gordon and Breach: New York and London, June 1970.) 210s (\$25.20) boards; 95s (\$11.50) paper.

SEVERAL recent multi-author volumes have discussed in detail a variety of aspects of biological membrane structure and function, but none has provided a balanced overall view of the field. One might have hoped therefore that a new book entitled *Introduction to the Study of Biological Membranes* would be an inexpensive general text for the enlightenment of the non-specialist. This text, however, is neither inexpensive nor of a sufficiently balanced coverage to fulfil this particular need. It is an introduction largely to physico-chemical studies of membranes.

The strength of the book lies in its valiant attempt to present in a simple manner some of the fundamental principles and practices on which studies of the distribution and re-distribution of substances in biological systems are based. The authors adopt the excellent practice of frequent reference to original papers which provide examples of the types of experiment under

discussion and this leads eventually to a very useful bibliography, occupying some forty pages, which includes the titles of the papers. Reference to this bibliography becomes absolutely essential in relation to the authors' attempts to summarize other aspects of membrane studies. Attempts at brevity frequently lead to unjustifiable finality in the assessment of experimental findings. Observations on isolated membrane components under far from biological conditions tend to be reported as demonstrating something fundamental about membrane structure or function whereas the experiment may simply indicate a possibility which has not and perhaps could not be demonstrated with the intact membrane or the intact biological system. The membrane field is full of relevant observations but with few indisputable facts about the structure or the mode of operation of the intact membrane.

The translation from the Spanish is far from perfect but the effect is more amusing than confusing. Argentinian authors and East German printers might be excused for having overlooked such imperfections, but New York and London publishers ought to make some effort to ensure the quality of their English texts.

J. B. FINEAN

THE BRAIN INTRODUCED

The Brain

Towards an Understanding. By C. U. M. Smith. Pp. 392+18 plates. (Faber: London, May 1970.) 90s.

THIS book is aimed at "the educated layman", sixth-form scientists and perhaps beginning science undergraduates. It should therefore be criticized for its interest and general appeal rather than for its academic excellence. The book is clear, although wordy and full of tautology. "As we have seen" sometimes occurs in one guise or another in three or four successive sentences, while solecisms like "We all of us" are distressingly frequent. While these stylistic mannerisms irritate me, I have been told by non-specialists who have read the book that the expository style in general is effective and interesting. Coverage of the literature is excellent, although often uncritical; so, although I have learnt from the book, I have also been annoyed and disturbed by the easy acceptance of dubious concepts such as "centres" in the hypothalamus and the storage and transfer of learned information by macromolecules.

It would be possible for me to point out many other inaccuracies and misstatements, but I feel that C. U. M. Smith's achievement is quite real and that his book does offer a comprehensive introduction to the major interests of those now doing "brain research". "The Brain; Towards an Understanding" is a presumptuous title. "The Brain: an Introduction to Current Research" might be more accurate, but the book as it is deserves success, though it is in no sense a critical text.

ANTHONY ROBERTSON

AUSTRALIAN INSECTS

The Insects of Australia

A Textbook for Students and Research Workers. (Sponsored by the Division of Entomology, CSIRO, Canberra.) Pp. xiii+1029. (Melbourne University: Melbourne, July 1970.) 185s; A\$19.80.

It is now more than forty years since R. J. Tillyard wrote *The Insects of Australia and New Zealand*, a book that has long been out of print. The present volume, sponsored by the Division of Entomology, CSIRO, under the general editorship of Dr I. M. Mackerras, is not to be considered as a revised version but is intended to be a textbook of entomology in its own right, with emphasis

on Australian insects, and in this it succeeds admirably.

A number of textbooks and treatises on entomology have been written in recent years from a non-taxonomic point of view, discussion of insect classification being relegated to a short chapter at the end. The present volume, however, has maintained a strong taxonomic treatment throughout and follows along the general lines of Tillyard's classic and the British textbook by A. D. Imms. It is always helpful for students to understand thoroughly the natural orders into which insects are divided before undertaking research along other lines and so it is refreshing to see an entirely new book written once again in a taxonomic style, though perhaps this was inevitable because so many of the authors are working taxonomists.

The general layout of the text follows that of other similar books, the first 200 pages covering anatomy, physiology, general biology, and the like while the remaining 800 pages are devoted to authoritative accounts of the insect orders. A good feature is that each of the thirty-seven chapters has been written by a specialist on that particular subject; half of the thirty authors are staff of the CSIRO, and the remainder are chiefly well known Australian entomologists. In very few cases has it proved necessary to seek authors elsewhere. Interesting chapters in the first part deal with the fossil record and the composition and origin of the Australian fauna.

One of the most striking features is the large number of figures, many of whole insects, the majority being new figures drawn for this book. No expense has been spared to ensure that everything is clearly and fully illustrated. All the figures are good, many are very good, those by Mr Frank Nanninga being especially outstanding. Mr Nanninga has also contributed the eight coloured plates.

It might be argued that there are enough textbooks of this kind already published, but most are some years old and are written principally with holarctic insects in mind. A glance at this one will show it to be a major step forward in the study of insects, if only in that the examples chosen are almost invariably Australian species. In fact, it contains a mass of information of a kind often difficult to find in the literature. For instance, included under each insect order is a modern classification to family level with a discussion and keys, frequently original, to the families known to occur in Australia. A short account is given of each of the latter families. The result is a valuable book that will undoubtedly be much in demand throughout the world.

PAUL FREEMAN

EXODUS FROM THE SOIL

Réactions des Microarthropodes aux Variations de l'État Hydrique du Sol

Techniques Relatives à l'Extraction des Arthropodes du Sol. (Programme Biologique International. Recherche Cooperative sur Programme du CNRS, No. 40.) Pp. 319. (Editions du Centre National de la Recherche Scientifique: Paris, 1970.) 62.35 francs.

THIS volume consists largely of two papers by Dr Guy Vannier, preceded by an explanation by Professor Delamarre-Debouteville of the purpose and organization of the CNRS programme No. 40 on the ecology of the soil.

Vannier's first paper describes the reactions of small arthropods to variations in soil moisture; in this he demonstrates that there are characteristic levels of water deficit (pF) at which two principal groups of species move out of the soil as it dries out. These are about 4.2 (the wilting point of higher plants) and 5.0 (considerably drier) for the isotomid Collembola and the oribatid mites respectively.

The paper contains: (1) A field study involving seasonal sampling of arthropod numbers and soil moisture in which it is shown that the soil moisture in the woodland study areas near Paris was always too high to affect faunal distribution. (2) A laboratory study using a type of weighing oven in which thin slices of soil are dried above a funnel which leads to a fraction collector. The water loss curve is related to the pattern of exodus of the invertebrates and their movements correspond with the pF values mentioned earlier. (3) A field study in which change in vertical distribution of the arthropods in control samples is compared with that in a large "wind tunnel" erected over a 5 m² plot. Both absolute numbers and vertical movements correspond with the same pF values.

The second paper attempts to apply the above findings to the design of arthropod extractors. A survey of such devices is followed by the description of one which is based on the weighing oven device; it is claimed that herein lies the basis for a highly selective extractor. Thin soil slices surrounded by warm dry air do not develop vertical physical gradients and the gradient approach to extraction techniques is hardly considered; nor is the role of temperature (apart from its general effect on mobility). The use of radio-frequency heating in Tullgren type extractors is reported but the results do not seem to improve on existing methods.

This very thorough investigation undoubtedly establishes the levels at which desiccation influences arthropods to leave soil; the demonstration of vertical movement in response to desiccation is valuable and the review of techniques in French will doubtless be useful to Vannier's countrymen. One is left with the impression, however, that were the IBP truly international, Vannier's work could have been more usefully and concisely coordinated with that on arthropod extraction which has been going on in other countries.

AMYAN MACFADYEN

BLISTER BEETLE TAXONOMY

The Bionomics of Blister Beetles of the Genus *Meloe* and a Classification of the New World Species

By John D. Pinto and Richard B. Selander. (Illinois Biological Monographs, No. 42.) Pp. 222. (University of Illinois: Urbana, Chicago and London, June 1970.) 85s.

BEETLES of the family Meloidae have attracted human interest since classical antiquity, on account of the medicinally active cantharidin which can be extracted from their bodies. Regrettably, modern rules of zoological nomenclature have had the result of establishing the names *Cantharis* and *Cantharidae* for beetles quite unrelated to Meloidae, and not yielding cantharidin. In the past two centuries, entomologists have discovered the remarkable parasitic life-cycles of Meloidae, and during the past two decades Professor R. B. Selander has built up a school of studies on the family in Urbana, devoted particularly to the habits and classification of the North American species. This well illustrated study of the genus *Meloe* is one of a series of similar ones devoted to various Meloid genera, produced by the Urbana school. It should be of particular interest to British entomologists, in that two or three species of *Meloe* are fairly common, conspicuous and easily recognized British insects; though Pinto and Selander do not provide any fresh information on the British species, they deal with some very closely related American forms.

One of the most interesting features of this and other works of Selander's school is the way in which secondary sexual structural features are shown to be functionally related to particularities of behaviour, and behavioural features are shown to have systematic importance much as they have been in gulls by Professor Tinbergen. This

aspect of the work will delight all true Darwinists. Information on the behaviour and habits of the parasitic larval stages is much harder to come by than that on adults, but Pinto and Selander record interesting observations in the course of laboratory rearing of *M. dianella* and *M. laevis*, using pollen, honey and hive-bee larvae as food.

From the point of view of classification, perhaps the most notable innovation in the work is the re-definition of the tribe Meloini to include the North American *Spastonyx*, whose adults are fully winged, look very unlike the heavy flightless *Meloe*, and have hitherto been placed in Lyttini. The first instar larvae of *Spastonyx*, however, are very like those of *Meloe*, and probably have similar phoretic habits. It is to these phoretic habits of the "triungulins" that the authors attribute the wide distribution of *Meloe*, in spite of the flightless adults; distribution patterns in *Meloe* probably reflect those of their bee hosts.

This book might well stimulate some student in a British university to undertake studies similar to those of Pinto and Selander of our native *Meloe* and will certainly be invaluable to anybody needing to identify the American species. A small criticism is that the authors have misleadingly treated as analogous phenomena the attraction to cantharidin of *Atrichopogon* flies and of certain anthicid beetles.

R. A. CROWSON

COUNTRYSIDE SOUNDS

Highland Birds

(BBC Wildlife Series No. 4.)

Wildlife of East Anglia

(BBC Wildlife Series No. 7.)

Selected and introduced by Eric Simms. (BBC Radio Enterprises: London, 1970.) 21s 6d each.

THESE two long-playing disks present the more spectacular voices of two regions which attract ornithologists each year in increasing numbers. *Highland Birds* concentrates on Strathspey and Cairn Gorm where conditions approach most nearly those of its far north, from pine forests to arctic-alpine hilltops, with a final band devoted to the islands. Altogether some seventy species make a vocal appearance and the effect is therefore somewhat that of a whistle or song-stop tour with Eric Simms as well-informed commentator. If the purpose of the record is to arouse happy memories or stimulate more birdwatchers to visit these exciting areas, the method used is admirable; but those who wish to learn the songs and calls of these rare birds may be a little disappointed: each makes a relatively brief contribution. The treatment is broadly ecological, each band covering a different major habitat, though inevitably there is some overlapping. The finale is a comparison between the songs of four races of wren *Troglodytes troglodytes*, three of them associated with island groups, rounded off by the voice of Shetland's own *Sturnus vulgaris zetlandicus*.

Wildlife of East Anglia is presumably so called because short recordings of frogs, toads and coypus are included; but all the other voices are of birds, over seventy species from occasional rarities like the two-barred crossbill *Loxia leucoptera*, to the naturalized Egyptian goose *Alopochen aegyptiacus*. But all the famous broadland voices are there too; so are those from the Ouse Washes, recently colonized by ruff *Philomachus pugnax* and black tern *Chlidonias niger*, though the former is not heard.

In choosing his material Eric Simms has endeavoured to use as much as possible from recordings made in the areas described. But he has not hesitated to draw where necessary on the pick of post-war tapes, and the great master Ludwig Koch is among the contributors. The

information given, like many of the recordings, is right up to date and the jackets carry full annotations, together with an introduction by Mr Simms.

BRUCE CAMPBELL

NO BONES ABOUT POLYMERS

Polysaccharide-Protein Complexes in Invertebrates

By S. Hunt. Pp. v + 329. (Academic: London and New York, March 1970.) 100s.

THIS is a careful compilation of facts in an area where facts are short and the connoisseur of polysaccharide structure will find it fascinating. Molecules are portrayed which have the interest of the flora and fauna of a newly discovered Australasia—so strangely the same, yet so strangely different, compared with other continents of the kingdoms of life.

It is very much the book of a biologist, and a chemist's susceptibilities are often offended. He is left to make what he can, without explanation, of materials which are "PAS positive" (p. 35), or emerge from "Sevag treatment" (p. 37) or "treatment with Lloyd's reagent" (p. 21), and so on throughout the book. Perhaps he misses the point by pausing to ask for a literal translation of each ritual phrase? There is excessive discussion of technique, and minor experimental details are recited as if they came down from Sinai on tablets of stone rather than being, so often, arbitrary. It is difficult to remain calm when glycosaminoglycan types are discussed as if each structure is as well defined as benzoic acid or methane, and when homage is paid to my own *bête noire*, the "calcium bridge" (p. 107). Alternative molecular structures are earnestly presented when there is little foundation for any (for example, pp. 107–108). The speculation about heteropolysaccharide biosynthesis, in a key part of the book (p. 290), is not informed by the knowledge since 1965 of such systems in bacteria. Indeed, the derivatized chitin of bacterial cell walls is not mentioned, even though it might be a "missing link" that the author seeks in molecular evolution.

Compensations do, however, follow from this biological perspective. Chapter sixteen is an exciting discussion of the evolution of polysaccharides, and there are profound thoughts about polysaccharide function throughout the book. These provide a refreshing change from the lame teleological arguments that are furtively aired by chemists and by chemical and physical biochemists. This book ought to be widely available, and deserves to be much read and discussed by those working in related research areas.

D. A. REES

IDENTIFYING BLOODSTAINS

La Recherche des Antigènes Gm dans les Taches de Sang pour leur Identification Biologique et Médico-légale

By Robert Görtz. (Centre National de la Recherche Scientifique. Monographies du Centre d'Hématologie, CHU du Toulouse.) Pp. 131. (Hermann: Paris, 1970.) n.p.

THE contents of this monograph are not confined to the subject indicated by the title. The historical and theoretical aspects of the Gm system are considered at some length but no significant information emerges which is not available elsewhere. The identification of other factors in bloodstains is also discussed, but, apart from somewhat cursory mention of absorption-elution, mixed agglutination and fluorescent antibody techniques, this section is devoted almost entirely to the use of the classical absorption-inhibition method for the detection of red cell antigens and is therefore far from comprehensive. There is also a brief chapter on the use of Gm typing for paternity testing.

In dealing with the detection of Gm factors in dried bloodstains an account of previous work on the subject is given. Attention is drawn to the fact that, because of the large number of antigens in the system, it would be possible to obtain a more precise identification of stains than by the red cell factors providing the quantity of stain was sufficient. (This is obviously assuming availability of the full range of suitable Gm typing sera.) The method for typing fluid serum samples is given, together with the adaptations for working with dried bloodstains. These consist of substituting pieces of bloodstained material, powdered blood or saline extracts of stains in place of the serum samples.

Various factors are considered which could influence the results obtained from stains. These include the type of serum, time and temperature conditions for absorption, the quantity and the age of bloodstains. Most of the work was carried out in respect of antigens Gm (1), Gm (2) and Gm (5). Original work is also reported on the identification of Gm (3), Gm (6) and Gm (10). A remarkably high standard of reliability is claimed even with stains which were several years old. Very detailed results are given and there is an abundance of tables, graphs and histograms.

A comparison is also made of serum samples and bloodstains obtained from newborn infants and their mothers. It is shown that, at birth, the baby has an identical Gm phenotype with that of its mother.

MARGARET PEREIRA

SULPHUR BIOCHEMISTRY

The Biochemistry of Inorganic Compounds of Sulphur

By A. B. Roy and P. A. Trudinger. Pp. xv + 400. (Cambridge University: London, June 1970.) 120s; \$18.50.

THE complexity of the field of sulphur biochemistry reflects the reactivity of the element itself and anybody with even the scantiest knowledge of the subject will readily appreciate that the authors of this book were faced with a formidable task. For the first time an attempt has been made to bring together in a single volume knowledge from various branches of the subject; knowledge which would be of value both to the general reader and to the specialist who is seeking a work of reference. The authors have attempted to dissociate the subject matter from the more familiar aspects of organic sulphur biochemistry. The distinction, however, between inorganic and organic compounds of sulphur is rather tenuously defined and to some readers the title of the book may seem to be misleading.

In thirteen chapters the authors cover the chemistry, enzymology, metabolism and biological significance of a wide range of compounds including simple molecules such as sulphide, sulphite, sulphate, thiosulphate and dithionate, and relatively complex compounds such as mustard oil glycosides, sulphate and thiosulphate esters, sulphamates, sulphatophosphates, sulphonic acids and steroid sulphatides. In the earlier chemical chapters attempts are made to relate structure with chemical reactivity and methods for the preparation and analysis of some of the more important compounds are described. In this last respect the book tends to fall between two stools because the treatment is certainly not sufficiently comprehensive for the specialist and may be considered superfluous by the ordinary reader.

A large section of the book is devoted to the biochemistry of the sulphate ion and deals with the enzymatic activation of sulphate, its transfer by way of sulphotransferase enzymes, to various types of acceptor to yield sulphate esters, and the hydrolysis of these esters by sulphatases. Much of the material presented has previously been reviewed by various workers, but the

book would have been incomplete without it and some newer aspects of the subject are brought to light.

The oxidation and reduction of inorganic sulphur compounds by plants, microorganisms and animals, transformations vital to the continuing re-cycling of sulphur in nature, are described in detail in later chapters. These processes set the stage for the concluding chapters of the book which constitute a novel attempt to appraise the biological significance of inorganic sulphur compounds from both a medical and an economic standpoint.

These various approaches serve to emphasize the important role of sulphur biochemistry to life on this planet and the book represents a most useful first attempt to gather together information concerning a very diverse field of activity. To cover the subject comprehensively would have been an impossible task in anything less than an advanced treatise and not everybody will agree with the emphasis placed on different aspects by the present authors. On the whole, however, they have succeeded in producing a very readable text which will certainly be welcomed by research workers in this particular field and which could be regarded as compulsory reading for anybody contemplating entering the field for the first time.

K. S. DODGSON

CAPRICIOUSNESS UNDERSTOOD

The Science, Technology and Application of Titanium
Edited by R. I. Jaffee and N. E. Promisel. (Proceedings of an International Conference held in the Royal Festival Hall, London, May 21-24, 1968.) Pp. xxi+1202. (Pergamon: Oxford, London and New York, May 1970.) 360s; \$48.

THIS volume comprises the proceedings of an international conference on titanium which needed the spaciousness of the Royal Festival Hall to accommodate the numerous participants. It represents the fourth milestone in the history of the titanium industry. The first was erected in 1938 by Dr Kroll when he attempted to attract commercial interest in the ductile titanium that he had produced by magnesium reduction of the chloride. Since then, at regular ten yearly intervals, widely supported scientific gatherings have assessed the state of titanium development and application. This conference is the third of these and was organized jointly by the Institute of Metals, the Metallurgical Society of the AIME and the American Society of Metals, in association with the Japan Institute of Metals and the Academy of Sciences of the USSR.

Kroll refers to titanium as "this capricious metal". The adjective may have seemed particularly apt to those who, like him, wrestled in the early days with the unfamiliar problems presented by the metal's extreme reactivity and the first encounters with hydrogen embrittlement. But in the short span of three decades titanium and some of its alloys have reached the point where at least their capriciousness is sufficiently well understood to allow them to be used with confidence where the high strength to weight ratio and special corrosion resistance meet service conditions better than other materials. It might be regarded as a sign of the attainment of its maturity that Rolls-Royce fell back on a titanium alloy for the fan blades of the RB 211 engine when 'Hyfil' became suspect because of its inadequate impact resistance. The general impression from the papers presented at this conference is of a phase of consolidation. There are many scientifically competent tidying-up operations but nothing startlingly new. The views held some time ago are confirmed that the problems arresting titanium development are in the realms of physical metallurgy, manipulation and understanding the behaviour in real engineering situations. Problems of extraction and refining are few and, indeed, were outside

the terms of reference of the conference. Stress corrosion cracking and hydride embrittlement are still a source of preoccupation as is corrosion in the presence of halides. But little is said about the important problem of scrap recycling, of development of powder metallurgy techniques for alloy production or of the grievous tribological problems that titanium generates.

All in all, the volume communicates an impressive account of advance in the science and technology of titanium during three decades. It confirms the arrival of titanium as an important weapon in the armoury of the materials technologist, but emphasizes that its further development, as with sales of the present volume, will be restricted by its high cost per pound. Reference libraries, however, will need to have this book as an authoritative account of the current state of the science and technology of titanium. Aspiring Titans who wish to gauge the current climate of opinion would do well to read first the remarks of the rapporteurs who introduced the various groups of papers and the ensuing reported discussions which are particularly revealing and valuable.

K. M. ENTWISTLE

SURFACE IMPURITIES

Clean Surfaces

Their Preparation and Characterization for Interfacial Studies. Edited by George Goldfinger. (Based on a Symposium held at North Carolina State University at Raleigh.) Pp. xix+385. (Dekker: New York, April 1970.) 178s; \$18.75.

It is well known that most surfaces are more or less covered with adherent layers of other adventitious materials—adsorbed gas, liquid or solid layers, oxide or other corrosion layers, or particles such as dust, oils or sediments which have simply settled and adhered. Such layers can strongly affect the physical and chemical properties of a material, and are clearly important.

This book consists of a collection of papers presented at a conference. The result is a rather mixed group of topics which cover a wide range of materials, and it is the accounts of refined modern techniques for detecting and assessing small traces of surface impurities, even of monatomic layers or less, that make this book a useful and instructive addition to the literature.

In general, it can be said that high purity is most difficult to achieve in complex organic materials such as polymers, and the first three chapters, on polyethylene, latexes, and blood/synthetic polymer interfaces, are therefore particularly important and interesting. Chapter four outlines clearly ellipsometry as a very sensitive tool for characterization of surfaces, and chapter five (by H. E. Farnsworth) gives some recent new results illustrating procedures which give much detailed information on the cleanness of metal single-crystal surfaces in ultra-high vacuum. Chapter six illustrates the now well known and appreciated techniques of scanning electron microscopy, which is important because it can be used to examine actual surfaces instead of replicas, with great depth of focus and resolution down to about 200 Å. The next four chapters deal with adsorption in gases and in solutions, involving considerations of typical data and present theories. Chapters eleven and twelve are on electrochemical techniques; and chapter thirteen is an extensive and informative account of techniques and criteria (surface tension, wetting, and the like) in the purification of aqueous solutions. The last chapters cover leached glass surfaces; pretreatment of mineral surfaces and its effect on their properties; the electronic surface states of finite lattices; impurity concentrations at "clean" oxide surfaces; and the detection and control of organic contaminants on surfaces, for example in the laboratory air, or in storage conditions.

As a whole, the book is well produced, clearly printed and well illustrated, and contains useful lists of references. The contributions from many experts in famous laboratories are authoritative and make this a valuable and helpful book.

H. WILMAN

INTRICATE LANDSCAPE

The Study of Landforms

A Textbook of Geomorphology. By R. J. Small. Pp. 486. (Cambridge University: London, July 1970.) 90s; \$14.50.

THIS textbook on geomorphology is interesting for its selective content and methodological intentions. It makes no attempt to give a comprehensive range of landform studies and many aspects such as vulcanism, mountain building, continental drift and deltaic deposition, which most authors would consider fundamental to the subject, are deliberately omitted. These omissions have allowed space for greater detail on topics such as the cycle of erosion, slope development, planation surfaces and periglacial landforms, which are treated at commendable length for an introductory text.

The aim is to present methodological and observational problems in a way that reveals the intricacies and uncertainties of landform analysis within a framework of reality based on local knowledge or fieldwork. Thus the volume is geared closely to the detailed working out of a few examples situated, except in the sections on tropical and sub-tropical landforms, chiefly in southern Britain, the Isle of Arran, the Grands Causses of southern France and the Val d'Hérens in Switzerland. In the author's view, a real knowledge of a few areas, which may or may not contain all the so-called "typical" landforms, is infinitely preferable to a wider and vaguer knowledge of many examples.

The effect on the text is strikingly apparent. Of the 200 illustrations, more than fifty deal with southern England. Some of the typical landforms given are excessively parochial and seem to be dragged in because they are readily accessible to fieldworkers from southern schools and colleges. Thus for Jura-type relief we are taken to east Hampshire where the present relief results from river-erosion working on a surface of marine planation and etching out valleys in the Tertiary sediments preserved within the chalk synclines, leaving the resistant chalk ridges upstanding. Undoubtedly, these regional case studies ensure that the text is very illuminating on certain aspects of geomorphology for anybody well acquainted with or living or doing fieldwork in southern Britain. Other readers may find some chapters overcrowded with unimportant localities and rather meaningless geological horizons. None will carp at the author's emphasis on accurate observation and measurement, but some may wonder, to misquote Kipling, "what do they know of England who only England know?"

The text, which is designed for advanced work in schools and introductory courses in colleges and universities, is well produced, praiseworthy free of textual errors and enhanced with crystal-clear diagrams. The general standard of exposition is high but, almost inevitably, the complexities of landform analysis induce occasional uncertainties. For example, the gentle dip of Cotswold strata used in arguments on outlier formation is contradicted in a diagram; the solution rate of all gases does not increase with temperature; Davis did not consider "stage" as length of time; the "classic" work of Deperet on raised Mediterranean shorelines has, alas, proved illusory. Further, the bibliography, which will be useful to university students, will remain inadequate as long as fine, recently revised summaries of geomorphology by W. D. Thornbury and B. W. Sparks are omitted.

ROBERT P. BECKINSALE

Short Notices

The Prehistory of Africa. By J. D. Clark. (Ancient Peoples and Places.) Pp. 302+48 plates. (Thames and Hudson: London, July 1970.) 50s.

AFRICA is unique among continents, as far as is known in having a human prehistory which extends back as much as two and a half million years or even more. There are still many gaps in the record, particularly in the early stages of man's physical and cultural evolution but so much material has been recovered in the past ten to fifteen years, that it is good to have this readable and up to date review of current knowledge about the evolution of toolmaking and the sophistication of human societies in Africa. Students will benefit most from the volume: it contains abundant references to books and original papers for further reading, and is well supplied with photographs, maps and line drawings of tools ranging from the most primitive Oldowan to Neolithic industries. Anybody without some previous archaeological background may, however, find the book hard going; it is a pity that there is no glossary of terms, no complete geological time scale, no explanatory drawing of human skeletal anatomy, and no description of the methods of radiocarbon and potassium/argon dating.

A Field Guide to Rocks and Minerals. By F. H. Pough. Pp. xv+349+46 plates. (Constable: London, July 1970.) 35s.

THIS book is in two parts; part one gives a brief instructive account of mineral properties and classification, indicating the rock types in which the better examples might be found—this is the only mention of rocks and the book is therefore not a field guide to them. The second part is concerned solely with mineral descriptions and more than 250 types are identified; most of them are illustrated, many in colour. The descriptions commence with the native elements, progressing through the non-silicates to the silicates. As a field guide the book would be of limited value, because it would not aid mineral identification—it contains no determinative tables of recognizable properties, such as hardness, crystal, form, colour and streak, which might have been expected from the title. It is, however, a useful reference book for the collector and is a convenient (12×19 cm) size.

Interacting Macromolecules: The Theory and Practice of their Electrophoresis, Ultracentrifugation and Chromatography. By J. R. Cann. (Molecular Biology: an International Series of Monographs and Textbooks.) Pp. ix+249. (Academic: New York and London, June 1970.) 117s.

THIS is a useful and timely offering, which deals crisply with the theory of transport of macromolecules in rapid association-dissociation equilibrium. As the author acknowledges in his preface, the work of Gilbert and his group in Birmingham has been responsible for opening a new dimension in sedimentation analysis, as well as partition chromatography and free-boundary electrophoresis. Many of the original papers lie buried in that musty cellar of the literature, *Proc. Roy. Soc.*, and the field as a whole has until now been nowhere properly reviewed. Equilibrium methods such as sedimentation equilibrium and osmotic pressure have, however, not been included. The all-embracing title is therefore misleading, but readers interested in the application of transport theory will find the book a good investment. There is a chapter by Goad, which gives a Fortran program for the calculation of parameters from boundary profiles.

Correspondence

Dust-up in Space

SIR,—Now that an extension of interstellar extinction curves into the vacuum ultraviolet has been made possible by rocket¹ and satellite² observations the tendency has been to “duplicate” these curves with increasingly complex mixtures of scattering particles. Claims have been made that a fit between theoretical and observed extinction curve provides justification for a particular grain model. A good case in point is the recent paper “Interstellar Extinction by Graphite, Iron and Silicate Grains” (*Nature*, **227**, 51; 1970). Here, an attempt is made to fit the extinction curve over the wavelength range $0.5 \leq \lambda^{-1} \leq 7 \mu\text{m}^{-1}$ with Mie scattering by a mixture of three types of particle. Such agreement as exists must, for the following reasons, be considered as fortuitous.

(i) The refractive index of the silicate grains is taken to be constant at $m = 1.66$ over the entire wavelength range. The refractive index of iron is taken as constant for $\lambda^{-1} > 4$.

(ii) Grains are unlikely to be the perfect spheres assumed. For other than spherical particles, the Mie theory must be modified. A good experimental verification of the importance of grain morphology on extinction has recently been published by Lefevre³.

In these and other calculations a fundamental point appears to have been missed. This is that the only parameter that characterizes these particles apart from their “radius” is their refractive index and its dependence on wavelength. By using constant refractive indices any working back from an extinction curve to infer the presence of a particular type of material is useless. The refractive index $m = 1.66$ no more characterizes “silicates” than it does “hydrocarbons”. Surely if one is asked to believe that this result provides any sort of confirmation of the graphite-iron-silicate hypothesis one must demand that the calculations be done with realistic refractive indices.

Perhaps the only feature that does hold some clue to the nature of the material producing this extinction is the hump observed near $\lambda^{-1} = 4.6 \mu\text{m}^{-1}$. Here one should remember that although the height of the hump is uncertain and may vary from region to region its wavelength is well established. Despite the fact that Wickramasinghe and Nandy’s curves pass through the error bars on the data, their curves clearly peak at $\lambda^{-1} \approx 4.0 \mu\text{m}^{-1}$. Certainly no identifications can be justified on the basis of such a simplified model and such a great mismatch between theory and experiment.

Yours faithfully,

W. W. DULEY

York University,
Toronto, Canada.

¹ Stecher, T. P., *Astrophys. J. Lett.*, **157**, 125L (1969).

² Code, A. D., *Pub. Astro. Soc. Pacific*, **81**, 475 (1969).

³ Lefevre, J., *Astron. Astrophys.*, **5**, 37 (1970).

Monsters and Poltergeists

SIR,—I write to comment on your leading article “Monsters and Poltergeists” (*Nature*, **227**, 215; 1970) and to correct what I think are a number of misconceptions contained therein.

First, you stated that “professed belief in the Loch Ness Monster is probably strongest amongst those with stakes in the Scottish tourist trade”. It is simply not true that those involved with the tourist trade are the greatest protagonists; anyone at all familiar with the Scots (as I think I, as a native, may fairly claim to be) would know that believers and sceptics are evenly distributed in all walks of life. Indeed, the Provost (or perhaps now former Provost) of Inverness, a town which gains a large amount of trade from the publicity of the monster, is one of the leading sceptics.

Second, you imply that the paucity of sightings, as compared with the number of flying saucer reports, is counter-evidence to the monster’s existence. I know of no principle of scientific method which states that if a thing is only seldom observed, it does not exist. In any case, it is grossly unfair to compare numbers of sightings in the 25 square miles of Loch Ness with sightings in the whole of North America.

Third, you accuse the Loch Ness investigation of being “no more a scientific endeavour than the hunting of the snark”, and you contrast this investigation to the psychic investigators. The difference between their methods is surely one of necessity: the psychic investigators’ material can be brought into the laboratory. But the Loch Ness investigation’s subject is a lake, and the largest in Britain besides. I defy even the editor of *Nature* to investigate Loch Ness in his laboratory. But because the investigation conducts its research in the field, that is no reason for condemning it as unscientific.

Fourth, you say that “it should not be long before Unidentified Swimming Objects are sighted in the Scottish lochs as regularly as the reports of UFOs in the United States”. In reality, one of the most significant facts is that reports of “monsters” do not come from all lochs, but from a few geologically similar lakes in Scotland, Canada and Scandinavia.

Finally, you say that the investigation has not produced “any shred of monster”. If by that you mean an actual specimen, that is true. But one does not disbelieve the existence of stars because one has no samples: one believes the photographic evidence for their existence. And in its seven years, the investigation has produced many pieces of photographic evidence for the existence of something inexplicable in Loch Ness.

That “something” may not be a monster, but, contrary to what you suggest in your article, the investigation would be very happy to have what you call “a natural explanation for the sightings” (even though the tourist trade might not). But to deny the evidence, and to sneer as your article does at attempts to investigate it, is reminiscent of the French Academy’s eighteenth century scorn of the idea that stones could fall from the sky.

Yours faithfully,

JAMES M. PIGGINS

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Obituaries

Dr Heinz London

HEINZ LONDON, who died on August 4, was distinguished by the number and quality of the contributions he made in a wide field of physics and especially in the field of low temperature research. Born in Germany in 1907, he studied first at Bonn University and the Berlin Technische Hochschule. At Breslau University, under Professor (later Sir) Francis Simon, he first became interested in the electrodynamics of superconductivity, and investigated the high frequency resistance of superconductors. In 1934 he joined Simon at the Clarendon Laboratory and continued his work on superconductivity. In a letter to *Nature* he suggested a relationship between current and electric field which took account of the possible presence in a superconductor of normal conducting electrons. This was the starting point for the electrodynamic theory of superconductors which he then evolved in collaboration with his brother, the late Fritz London. One of their joint papers, "The Electromagnetic Equations of the Superconductor", is regarded as a classic in this field of work. London wrote independently on the phase transition of superconductors in a magnetic field, and carried out experiments to show that a superconductor acquired surface charges in a similar manner to that of an ordinary conductor.

From Oxford he went to the Wills Laboratory at Bristol and succeeded in measuring the high frequency resistance of superconductors, thus demonstrating the presence of normal electrons and their gradual decrease with decreasing temperature. During this period London also suggested and took part in experiments on the superconductivity of thin films. The superfluid phenomenon of liquid HeII was becoming known and London carried out a simple experiment which showed that an attraction is exerted at a light vane by a heated plate immersed in liquid HeII. Analysis of this experiment led him to establish the thermodynamic relationship between the fountain effect and entropy in HeII.

During the war, London worked on the atomic energy project and developed a method of separating the uranium isotopes by ionic transfer. This he showed to be unsuitable for large scale separation and at the end of the war he was investigating the liquid thermal diffusion method. This interest in isotope separation was further extended when in 1946 he was given the task of devising methods for separating stable tracer elements which were urgently

required by the Medical Research Council for biological work. He concentrated on ^{13}C , and in view of the known difficulties in chemical methods decided to try the method of low temperature distillation. He designed a fractional distillation column and used carbon monoxide for the enrichment of ^{13}C and ^{18}O isotopes. In the course of this he came across a new effect of considerable theoretical interest, namely, the effect on vapour pressure of the symmetry of the molecule. The distillation column has now operated for long periods and supplies all the ^{13}C used, not only in Britain, but also in the United States.

London joined the Atomic Energy Research Establishment at Harwell when it was set up in 1946. During the first years he was concerned with the development of the centrifuge technique of isotope separation; later, after this technique had been superseded by the economically more attractive gaseous diffusion method, he suggested a way of removing nitrogen continuously from the enriched uranium hexafluoride in the diffusion plants and successfully demonstrated the method on an experimental scale.

While at Harwell he continued his interest in low temperature research and designed a liquid helium cryostat for use with the BEPO reactor for producing cold neutrons. He later became involved in a project to design a liquid hydrogen refrigerator system to feed a small flask in a reactor, a project which required extreme care in safety design and in system reliability.

In the last few years his technical work concentrated on three major technological projects. These were studies of thin film and filamentary hard superconductors, the development of the $^3\text{He}/^4\text{He}$ dilution refrigerator, and a study of force-free current and magnetic field distributions in solid superconducting spheres. Of these the dilution refrigerator has had the greatest impact, and, developed for commercial sale, it is now being used in various laboratories for giving extremely low steady temperatures and considerable heat removal rates. This is likely to rank as one of the truly important contributions to low temperature research, for it allows long duration experiments with heat dissipation to be carried out in the temperature range below 0.1 K.

In 1959 London was elected to give the first Simon Memorial Lecture: he chose for the title of his talk "Superfluid Helium". He was elected a Fellow of the Royal Society in 1961.

Announcements

University News

Dr R. N. Haward, visiting reader in the University of Manchester Institute of Science and Technology, has been appointed to the chair of industrial chemistry, **University of Birmingham**.

Dr Arthur T. Hertig has become professor of pathology at **Harvard University** and chairman of the Division of Pathobiology at the New England Regional Primate Research Center.

Dr Richard K. C. Lee, dean emeritus of the University of Hawaii School of Public Health, has been appointed executive director of the Research Corporation of the **University of Hawaii**, in succession to **Dr Robert Hiatt** who resigned at the beginning of this year.

Mr Harold P. Rooksby, a senior scientist with General Electric Company and co-editor of the *Journal of Applied*

Crystallography, has been appointed external professor within the Department of Ceramics, **University of Leeds**.

Dr Mark Guter, managing director of CJB (Projects) Ltd, has been appointed to the second chair of chemical engineering tenable at University College London. The following titles have been conferred within the **University of London**: professor of psychopharmacology, on **Dr Hannah Steinberg** in respect of her post at University College; professor of anatomy, on **Dr D. W. James** in respect of his post at University College; professor of theoretical physics, on **Dr T. W. B. Kibble** in respect of his post at Imperial College; professor of biochemistry, on **Dr A. P. Mathias** in respect of his post at University College; professor of computer science, on **Dr P. A. Samet** in respect of his post at University College; professor of information processing, on **Mr K. Wolfenden** in respect of his post at the Institute of Computer Science.

Appointments

Robert Dougall, senior BBC Television newsreader, has been nominated president-elect of the **Royal Society for the Protection of Birds**.

Dr Joseph E. Clark has been appointed head of the Office of Flammable Fabrics, **US National Bureau of Standards**.

Dr M. G. P. Stoker, director of research of the Imperial Cancer Research Fund, has been appointed a member of the **Council for Scientific Policy** in succession to **Professor G. S. Brindley** who has resigned.

The **Science Research Council** has announced changes in membership of the four SRC Boards: *Astronomy, Space and Radio Board*: **Dr J. T. Houghton**, University of Oxford; **Dr R. W. Pringle**, Nuclear Enterprises Ltd, Edinburgh; **Professor E. D. R. Shearman**, University of Birmingham, and **Professor D. W. N. Stibbs**, University of St Andrews, have been appointed to the board. **Dr E. Eastwood**, General Electric and English Electric Companies, will become chairman of the board, in succession to **Sir Bernard Lovell** who is retiring, as are **Professor R. L. F. Boyd**, **Professor W. H. McCrea** and **Professor R. O. Redman**. *Engineering Board*: **Professor P. B. Hirsch**, University of Oxford, and **Mr D. J. Lyons**, Ministry of Transport Road Research Laboratory, have been appointed. *Nuclear Physics Board*: **Professor K. W. Allen**, University of Oxford, and **Dr I. S. Hughes**, University of Glasgow, have been appointed. **Professor D. H. Wilkinson** has retired as chairman and is succeeded by **Professor J. G. Gunn**, University of Glasgow. **Sir Derman Christopherson** and **Professor R. J. Blin-Stoyle** have also retired. *Science Board*: **Professor S. F. Edwards**, University of Manchester, and **Dr J. Paul**, Beatson Institute for Cancer Research, Glasgow, have been appointed and **Professor E. W. J. Barrington** has retired.

Miscellaneous

Professor Albert V. Crewe, professor in the Department of Physics and the Enrico Fermi Institute, University of Chicago, has been named **Man of the Year in Research** by Industrial Research Inc.

A series of courses in mammalian, plant and insect cell culture arranged by the **Tissue Culture Association** will be held at the W. Alton Jones Cell Science Center, Lake Placid, New York. Participation is not limited to members of the Association or to holders of a specific degree. Further information can be obtained from **Dr Donald J. Merchant**, W. Alton Jones Cell Science Center, PO Box 631, Lake Placid, New York 12946, USA.

The following fellowships in chemistry for 1970-71 have been awarded by the **Ramsay Memorial Fellowships Trustees**: a general (British) fellowship to **Dr J. R. Airey** at the University of Cambridge; a Canadian fellowship to **Dr R. G. Macdonald** at the University of Cambridge; a Netherlands fellowship to **Dr W. P. Zeylemaker** at the University of Oxford; a New Zealand fellowship to **Mr B. L. Dickson** at Imperial College, London; a Spanish fellowship to **Dr F. Gonzalez Vilchez** at Imperial College, London; United States fellowships to **Dr R. P. Hanzlik**, **Dr J. F. Liebman** and **Dr J. R. Wiesenfeld** at the University of Cambridge. The fellowships held by **Dr D. B. Sheen** (Leeds), **Dr E. W. Colvin** (Glasgow), **Dr M. F. Tchir** (Royal Institution) and **Dr M. Koiwa** (Oxford) have been renewed.

Applications are invited from candidates with a PhD degree or equivalent qualification for two **Unilever European fellowships**. One fellowship will be awarded to a British citizen for work in a laboratory in continental Europe and one to a European for work in Britain. The fellowships, worth up to £1,700 per annum, are tenable for

one year and are available for research in biochemistry in any laboratory or institute. Further information can be obtained from the Executive Secretary, the **Biochemical Society**, 7 Warwick Court, London WC1R 5DP.

The **Beilby Medal and Prize**, administered from the Sir George Beilby Memorial Fund and consisting of a medal and a sum of money, is now offered annually. The award will be made to a British investigator in recognition of independent original work of exceptional merit in the fields of chemical engineering, fuel technology or metallurgy, and is intended as an encouragement to younger workers in these subjects. Further information can be obtained from the Convener of the Administrators, Sir George Beilby Memorial Fund, Royal Institute of Chemistry, 30 Russell Square, London WC1B 5DT.

ERRATUM. The following corrections should be made to the article by **J. J. Cazzulo**, **T. K. Sundaram** and **H. L. Kornberg** entitled "Mechanism of Pyruvate Carboxylase Formation from the Apo-Enzyme and Biotin in a Thermophilic Bacillus" (*Nature*, **227**, 1103; 1970): line 21 of the legend to Fig. 1 should read " MgCl_2 , 0.5; ATP, 0.125; acetyl-coenzyme A, 0.1; . . ."; in line 12 of the legend to Fig. 3, "75 mM" should read "75 μM ".

International Meetings

September 23-25, **The Organization and Social Implications of Science in Africa**, Aberdeen (Mr D. B. Capitanchik, Department of Politics, King's College, University of Aberdeen, Old Aberdeen AB9 2UB).

September 24-25, **Biochemical Society Meeting**, Dundee (The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 28, **Association for High Speed Photography Autumn Conference**, London (Mr R. J. Cox, Association for High Speed Photography, 44 Gade Avenue, Watford, Hertfordshire).

September 28-29, **Biochemical Society Meeting**, Norwich (The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

October 4-10, **International Astronautical Congress**, Constance (International Astronautical Federation, 250 Rue Saint-Jacques, Paris 5, France).

October 11-15, **The Information Conscious Society**, Philadelphia (Miss Sheryl Wormley, ASIS, 1140 Connecticut Avenue NW, Suite 804, Washington DC 20036, USA).

October 16, **Biochemical Society Meeting**, London (The Biochemical Society, 7 Warwick Court, Holborn, London, WC1R 5DP).

October 24, **Demonstration Meeting of the Palaeontological Association**, London (Dr Gwyn Thomas, Geological Department, Imperial College, London SW7).

November 9-21, **Agrometeorology**, Barbados (World Meteorological Organization Secretariat, Geneva, Switzerland).

November 16-18, **Cleansing**, Bournemouth (General Secretary, Society of Cosmetic Chemists of Great Britain, 56 Kingsway, London WC2).

Sabbatical Itinerants

Entries of this kind can now be found among the classified advertisements

British Diary

Monday, September 21

Electronic Engineering in Ocean Technology (four-day conference) Institution of Electronic and Radio Engineers, and the Institution of Electrical Engineers, at the University College of Swansea, South Wales.

Temperature Measurement (three-day conference) Institute of Physics and the Physical Society, in association with the Institution of Electrical Engineers; the Institution of Electronic and Radio Engineers; and the Institute of Measurement and Control, at the University of Warwick.

Tuesday, September 22

Adaptive Control of Machine Tools (6 p.m. discussion) Institution of Mechanical Engineers, jointly with the Institution of Electrical Engineers, and the Institute of Measurement and Control at 1 Birdcage Walk, London SW1.

Analysis and Control of Pollution (one-day symposium) Society for Analytical Chemistry, and the Bristol and District Section of the Royal Institution of Chemistry, at the School of Chemistry, University of Bristol.

Nuclear and Particle Physics (three-day Conference) Institute of Physics and the Physical Society, Nuclear Physics Sub-Committee, at the University of Oxford.

The Vitreous State (three-day discussion meeting) Faraday Society, at the University of Bristol.

Wednesday, September 23

Cost Accountancy in the Seventies (6 p.m. conference) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Critical Factors in the Application of Diesel Engines (two-day symposium) Institution of Mechanical Engineers; and the Diesel Engineers and Users Association, at the University of Southampton.

High Energy Physics (three-day conference) Institute of Physics and the Physical Society, Nuclear Physics Sub-Committee, at the University of Durham.

Stress Analysis Today (three-day conference) Institute of Physics and the Physical Society, Stress Analysis Group, at the University of Surrey, Guildford.

Thursday, September 24

Air Coolers (conference) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Anaerobes and Man's Environment (10.30 a.m.) Society of Chemical Industry, Microbiology Group, in the Botany Lecture Theatre, University College London, Gower Street, London WC1.

Analytical Chemistry—Industrial and Academic Training (two-day symposium) Society for Analytical Chemistry, Education and Training Committee, at Loughborough University of Technology.

Biosocial Aspects of Human Fertility (two-day symposium) Eugenics Society, in the Meeting Rooms of the Zoological Society of London, Regent's Park, London NW1.

Odour and Taste Measurement (7.30 p.m.) Dr D. G. Land, British Society of Perfumers, at the Royal Society of Arts, John Adam Street, Adelphi, London WC2.

What is a Mechanism? (6 p.m. discussion) Institution of Mechanical Engineers, Applied Mechanics Group, 1 Birdcage Walk, London SW1.

Friday, September 25

Colour Measurement (7.15 p.m.) Mr R. P. Best, Oil and Colour Chemists' Association, at the Royal Hotel, Bristol.

Determination of Electronics Energy Levels; The New Spectroscopic Techniques (informal discussion) Chemical Society, in the Inorganic Chemistry Laboratory, South Parks Road, Oxford.

Future Pattern of the Training and Employment of Biologists (9.30 a.m. conference) Institute of Biology, at The Royal Society, Carlton House Terrace, London SW1.

The Applications of Chromatography in Food and Agriculture (2.30 p.m.) Society for Analytical Chemistry, Chromatography and Electrophoresis Group and the East Anglia Section, at the University of East Anglia, Norwich.

Wood Protection (6.30 p.m.) Mr G. L. Holbrow, Oil and Colour Chemists Association, at the Chamber of Commerce House, 75 Harbourn Road, Birmingham 15.

Sunday, September 27

Cell Walls and Cell Membranes (Second Harden Conference, six days) Biochemical Society, at Wye College, Ashford, Kent.

Monday, September 28

Some Problems and Developments in the Field of Automotive Lubrication (6 p.m.) Mr A. Towle, Institution of Mechanical Engineers, Automobile Division, at 1 Birdcage Walk, London SW1.

Thin Liquid Films and Boundary Layers (three-day discussion Meeting) Faraday Society, at the University of Cambridge.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

- Regulations for the Electrical Equipment of Buildings. 14th edition. Reprinted in Metric Units incorporating amendments 1970. Pp. vi+223. (London: The Institution of Electrical Engineers, 1970.) 25s. [208]
- Lincolnshire Natural History Brochure, No. 5: Supplement to the Birds of Lincolnshire, 1954-1968. By the late R. K. Cornwallis. Edited and revised by K. Atkin and A. D. Townsend. Pp. 38. (Lincoln: Lincolnshire Naturalists Union, 1970.) [208]
- Bulletin of the British Museum (Natural History). Zoology, Vol. 20, No. 1: The Cluproid Fishes Described by Steindachner. By P. J. P. Whitehead. Pp. 1-46+3 plates. (London: British Museum (Natural History), 1970.) 29s. [218]
- The Natural Rubber Producers' Research Association. Thirty-second Annual Report. Pp. 45. (Welwyn Garden City, Herts: The Natural Rubber Producers' Research Association, 1970.) [218]
- Building Research Station. Current Paper 20/70: Initial Behaviour of Scammonden Dam. By A. D. M. Penman and P. B. Mitchell. Pp. 24. (Reprinted from Proceedings of the 10th Conference of the International Commission on Large Dams, Montreal, 1970.) (Garston, Watford: Building Research Station, 1970.) *Gratis*. [2218]

Other Countries

- Rhodesia. Report of the Trustees and Director of the National Museums of Rhodesia for the year ended 31st December, 1969. Pp. 31 (Salisbury: National Museums of Rhodesia, 1970.) [208]
- Organization for Economic Co-operation and Development. Gaps in Technology—Electronic Computers. Pp. 209. (Paris: OECD; London: HMSO, 1969.) 13.50 francs; 21s; \$3. [208]
- US Department of the Interior: Geological Survey. Bulletin 1293: Selected Annotated Bibliography of Minor-Element Content of Marine Black Shales and Related Sedimentary Rocks 1930-65. By Elizabeth B. Tourtelot. Pp. xv+118. \$0.60. Professional Paper 495-D: Structural Geology of the Wind River Basin, Wyoming. By William R. Keefer. Pp. iv+35+3 plates. Professional Paper 644-E: The Upper Part of the Upper Triassic Chinle Formation and Related Rocks, Southeastern Utah and Adjacent Areas. By Robert B. O'Sullivan. Pp. iii+22. \$0.40. Professional Paper 667: Stratigraphy and Nomenclature of Some Upper Cretaceous and Lower Tertiary Rocks in South-Central Wyoming. Pp. iii+53. \$0.65. Professional Paper 643-H: *Salterella* from the Lower Cambrian of Central Nevada. Pp. iii+7+5 plates. \$0.55. (Washington, DC: Government Printing Office, 1970.) [208]
- Tall Timbers Research Station Publications. Proceedings Annual No. 9—Tall Timbers Fire Ecology Conference, 1969. Pp. 292. (Tallahassee, Florida: Tall Timbers Research Station, 1970.) [208]
- US Department of the Interior: Geological Survey. Professional Paper 660: Geology and Mineral Resources of Libya—a Reconnaissance. By Gus H. Goudarzi. Pp. viii+104. (Washington, DC: Government Printing Office, 1970.) [208]
- US Department of Commerce: National Bureau of Standards. Research and Development in the Computer and Information Sciences. 3: Overall System Design Considerations—a Selective Literature Review. By Mary Elizabeth Stevens. (NBS Monograph 113, Vol. 2.) Pp. v+143. (Washington, DC: Government Printing Office, 1970.) \$1.25. [208]

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Will the Chickens come Home to Roost?

THE Science Research Council has become in the past few years one of the most clear-headed and outspoken of British public authorities, which is a great deal in its favour. The most recent annual report (see page 1284) is a striking proof that the council knows where it would like to go. Connoisseurs of documents like these will find themselves wishing that comparable institutions—the Natural Environment Research Council, which published its report only a week ago, for example—could borrow a little of the clarity that now marks the Science Research Council. It is also fair to say that much of what the council has chosen to touch in the past few years has turned to the grant-giving body's equivalent of gold—sensible sponsorship for imaginative research. In short, nobody will wish to quarrel with the details of what the council has attempted. Such disputes as there may be concern the larger issues, and in particular the framework within which the council has chosen to operate.

The record of the council's successes is worth reciting. In the first place, experience has shown that steps can actually be taken to make selected fields of research grow in directions that seem for intellectual reasons to be desirable. Some years ago, astronomy was singled out as a field in which extra efforts might be beneficial, and the result is that British astronomy has become a powerful contributor to European scientific life. It is especially cheerful that the Science Research Council has been willing to fall in with the wishes of many of the people concerned that British astronomy should acquire a cosmopolitan flavour. The hunt for observational sites abroad is one sign of the times (although it is unfortunate that the British involvement in the Southern Hemisphere with the Anglo-Australian telescope seems to have precluded British participation in the scheme to build a European Observatory in Chile). To be sure, there might be great benefits in a further attempt to make more sensible the present distribution of research between the several interested laboratories in Britain, but even here the past few months seem to have brought a great deal of improvement (of which the queues of astronomers clamouring to use the Isaac Newton telescope are a welcome sign). The thought, it seems, can indeed be the father of the deed.

Will the same techniques be equally effective in the other fields in which the council has resolved to concentrate its efforts? This is one of the questions to which some attention should urgently be given. To what extent are the factors responsible for the council's successful sponsorship of astronomy likely to be

replicated elsewhere? One immediate difficulty is that the sponsorship of astronomy came at a time when Britain seemed to be populated by people clamouring for funds with which to work. The development of radio astronomy in the fifties was partly responsible. So also was the growth of theoretical astronomy at several British centres. In less fortunate circumstances, people might simply have packed their bags and made off to the United States. With the best will in the world, there is no assurance that the areas of research in which the council has now decided to concentrate its efforts—enzyme technology, for example—will turn out to be equally well supplied with bursting talent. Indeed, the arguments which have led to the selection of these fields for research have often involved the calculation that the development of centres of activity at chosen universities is the most effective way of creating the pool of interest and talent. To point this out is merely to state the obvious, but it follows that the success of the council's policy of selectivity and concentration will depend much more crucially on the choice of the right people than its previous successes will have done. This is something to be reckoned with, but on the assumption that matters have been properly regulated, this is an issue on which the council deserves to be supported.

There is less certainty about the council's assumption—a part of conventional wisdom in British public administration—that the present division of labour between itself and the University Grants Committee is the best that could have been devised. To be sure, it is convenient for everybody concerned if the real conflict of interest which exists between the Science Research Council and the University Grants Committee can somehow be concealed beneath a layer of polite gentility, but this by itself does not ensure that such funds as are available are spent in the most effective way.

As things are and have been, the research councils are regarded as sources of financial support for research projects that are intrinsically novel. Theoretically, responsibility for established lines of research should pass from the research councils to the UGC. In the old days (five years ago) there used to be endless arguments about the exact timing of the process of handing over from one body to another, and there may yet again be ructions when the Council for Scientific Policy delivers its promised opinion on the issue. Whatever the intention, however, there is no doubt that the UGC is much less able than the research councils to be fierce in the application of the criteria for deciding when a research project deserves support. Not merely is there a necessary temptation to be impartial between departments and even universities, but it is obviously

harder to bring to a halt research activities which have been institutionalized within the framework of the UGC.

What should now concern the Science Research Council is the likelihood that the present arrangement for channelling through the UGC more than £40 million of research money every year is a brake on its capacity to select and concentrate. However reasonable may be the distinguished people who man the committees of the UGC, there is no doubt that the working of the system tends to favour diversity and diffusion, not the opposites. To say this does not imply malevolence—this is merely the way in which the systems work. In a sense, the Science Research Council (the most pushy of the research councils) may be lucky not to have been in headlong conflict with the universities long before this. But in the long run, there can be no doubt that its now declared policy would be more effectively applied if a much larger proportion than at present of what the universities collectively spend on research were to be handled by the research councils. In principle, there is no reason why everything should not be dealt with in such a way. It will be interesting to see how far the Council for Scientific Policy will go in this direction.

Another cloud on the horizon may soon be larger

still, for there is very little sign that the pressure on the council's moneybags will quickly be relieved. In retrospect, there is very little doubt that the council has been too complacent about the supply of money for research. The way in which it has had to support graduate students only in a fixed proportion of the graduating class in science and technology is a proof of its incapacity to respond flexibly to changing circumstances in the universities. In exactly the same way, the decision that the council could not afford to play a part in the building of the 300 GeV machine on which CERN had set its heart some years ago, even if not directly the responsibility of the council, should have been a warning that desirable projects were in danger of being abandoned for lack of money. One of the dangers in the present straitened circumstances is that the launching of some of the projects for selection and concentration will be hampered by the lack of money. Another is that the council may be prevented from playing the full part which it deserves in the impending argument which there must be about the whole pattern of undergraduate education at British universities. On both counts, there is an urgent need that the importance of the council's work should quickly be recognized by the new British government.

No SALT on the Tail

A MONTH from now, the negotiations between the Soviet Union and the United States on the limitation of strategic weapons will be opened in Helsinki. In the year since these talks began, the most important achievement has been the way in which both sides have learned to listen to each other's case. If the United States has so far done most of the talking, the explanation is probably the care with which the American negotiating position was spelled out at the beginning. If nothing emerges from the SALT negotiations, the whole proceeding will at least have taught western governments how best to set about preparing for international negotiations. That is something to be pleased about. The important issue is now, however, the extent to which the talks in prospect are likely to prepare the way for a more lasting agreement on strategic weapons. As yet, the Soviet Union seems to have given only the most imprecise hints of its probable reply to the American proposals, yet both sides seem anxious for an agreement of some kind. What stable basis can there be?

One of the inherent difficulties of principle in searching for an agreement along these lines is that of defining some characteristic of the strategic equipment now deployed in the East and the West which can be used as a basis for monitoring an agreement. At this stage, quite understandably, neither of the strategic powers seems to contemplate anything approaching total disarmament. Moreover, there is plainly no basis for an agreement for the abolition of all weapons in cer-

tain categories. A total ban on submarines, for example, would quickly be found unfair to one side or the other. Even attempts to abolish or prohibit multiple warheads on ballistic missiles would be frustrated by the difficulty of being able to tell from the appearance of the outside of a rocket whether the warhead is simple or complex. (For what it is worth, there seems to have been no suggestion, in the SALT talks so far, of a prohibition on multiple independent re-entry vehicles (MIRV) of the kind now being installed on some Minuteman missiles in the United States.) A prohibition on anti-ballistic missile systems is probably more feasible—these are, after all, recognizable for what they are—but now that the American case for building ABMs is linked with the case for diminishing vulnerability of rocket silos to attack at a distance, it is inevitable that any proposal to restrict the use of these devices would have to be traded off against a restriction on the numbers or the sizes of the long range ballistic missiles. So far as the United States is concerned, it seems as if one of the chief objectives is a limitation of the deployment of SS9 missiles in the Soviet Union.

What number of missiles on each side would be considered safe, however? One difficulty is, of course, that neither of the nuclear powers will willingly see its strength reduced to such a point that other powers—China, for example—might become in due course serious threats, which in turn implies that neither will willingly abandon the last five hundred missiles in its

armoury. But so long as weapons of some kind are to be kept, is there not a case for thinking, in the Soviet Union and in the United States, that there should be continual improvements of military technology with the objective of improving the efficiency of the striking force? In other words, no agreement based on numerical limitation alone is likely to diminish the interest of both sides in the advancement of military technology. Desirable though it may be to limit the actual deployment of strategic weapons, it therefore seems that the full benefits of SALT will only be attainable when some means has been found of limiting the nature of military research and development. How is this to be accomplished? Prohibitions on the testing of MIRVs and ABMs are unworkable, for who is to tell from a distance the difference between an advanced rocket and one of the kind now considered to be conventional? Even a complete cessation of the testing of ballistic rockets would be unworkable if only because the Soviet Union and the United States are only two of the countries that have set their hearts on vigorous programmes of scientific research in space. In the circumstances, the best candidate for an agreement that will reduce the incentives to military development in strategic weapons would be an extension of the test ban now in force to cover underground explosions as well as those which release radioactivity to the atmosphere. This is desirable in its own right, but is also plainly a desirable complement to a limitation on the deployment of strategic weapons after the manner soon to be talked about at Helsinki. If the great powers lack the wit to discover this simple truth for themselves, there is no reason why the United Nations Disarmament Committee at Geneva should not bring it forcibly to their attention.

Beyond the prospect, real or imaginary, of an early agreement at Helsinki, there lies the more difficult question of how the nuclear powers are to coexist for the indefinite future—at least a decade until mainland China is a power to be reckoned with and at least a decade after that until there is some hope that China can be brought within the negotiating community. The difficulty, of course, is that no amount of discussion at Helsinki in 1970 can predict with accuracy what military technologies will be in vogue in 1990. In circumstances like these, the important need is for some means of sharing and exchanging information between the two nuclear powers on the progress of military technology. In this spirit, no harm will be done if the first outcome of SALT is that the talks at Helsinki and Vienna become institutionalized. Like Pugwash, they have turned out to be most valuable because they are a way of showing each nuclear power that the other is also moderately reasonable. But there is also likely to be a continuing need for the more deliberate exchange of information about such things as the testing of long range rockets. Would it not be of some value if all flights of ballistic rockets above the atmosphere were registered voluntarily and accompanied by a declaration of purpose, military or peaceful as the case might be? The same is true of a great many of the activities in which the great powers are now engaged in exploration of the sea floor; these, too, are circumstances in which the availability of information would be a great reassurance for everybody concerned. In this sense, what matters most about the resumption of the SALT negotiations is that the two parties should agree to keep on talking. If there is no tangible agreement about which to boast, nobody needs to be downcast.

Responsibility without Choice

THE eloquent plea by Dr Philip Siekevitz (page 1301) that there should be a more explicit recognition of the rules by means of which the personal responsibility of scientists should be determined raises several important and interesting issues. Not the least of them is the sense in which the collection of all scientists can be regarded as a community of like-minded intellectuals who may be expected to accept a kind of ethical code—an analogy with oaths sworn in the old days.

Dr Siekevitz considers that scientists should not “engage in war work” and that those who “consistently use their skills in the service of killing men” should not be asked to scientific meetings and “should not be allowed to publish their results”, and in the process raises the even more teasing question whether it is possible to predict in advance just what the consequences will be of particular lines of scientific research. He goes on, fair play, to point out the difficulty of knowing what should be done about the more subtle problems of allocating responsibility for the consequences of more ambivalent developments—those which are at first

almost wholly desirable but which are later found to have unforeseen and unpleasant side-effects. His answer, in crude terms, is that the people concerned should see that they are more intimately involved in the process of decision making—then they will at least be unable to wash their hands of responsibility.

Of the main conclusion there is no doubt—in one way or another the scientific community should be more actively engaged in decisions about the social application of scientific work. On matters such as the use to be made of the supersonic civil aircraft, for example, technical people have a special contribution to make towards a sane decision. The difficulty, unfortunately, is that the most useful service which can be rendered is to clarify obscure issues. Rarely is there what might be considered as a consensus of scientific opinion. When it comes to balancing the advantages of carrying through a complicated technical development such as a supersonic transport aircraft against the evil but unpredictable effects of extra noise, with the added complication that nobody can be sure how the eco-

nomics will work out, there is nothing that can be called a scientific point of view.

Even the starkest issues usually turn out to be riddled with subtleties of this kind. Even military research is not an issue of black and white, for is it not entirely proper to consider national defence a kind of social duty? Or may it not at least be proper to consider the attainment of a condition of preparedness a praiseworthy goal? And what is to be said about those who have been working in the past few decades—pointlessly, perhaps—on the development of techniques for defending people against fallout? Would Dr Siekevitz decline to ask them to meetings, or would he ask for a standing ovation on their behalf, and sit them in the front row? These are also teasing questions.

100 Years Ago



The subject of Spontaneous Generation is undoubtedly the question of the meeting of the British Association for 1870. The title of the paper by Professor Huxley which headed yesterday's list in the department of Zoology and Botany, did not appear to bear directly upon it, and yet it was generally understood that it would reopen the subject. The President's discourse, for he had scarcely a note before him, was a popular account of the mode of development and form of those minute structures which the microscope reveals in such prodigious numbers in infusions containing organic matter, *Penicillium*, *Torula*, *Bacterium*, and *Vibrio*. He adduced arguments in favour of the theory that these various bodies are not distinct organisms, but are different modes of development of the same substance, and a more admirable and luminous exposition, it was generally admitted, has seldom been delivered. In the course of his remarks, Prof. Huxley took occasion to explain the difference between the "Brownian" motion of the molecules of inorganic matter, and the vital motions of living matter, and expressed his conviction that the motions observed by Dr. Bastian in the infusions which had been subjected to long-continued high temperatures, were referable to the former and not to the latter cause. During the discussion which followed, Dr. Bastian entered the room, but when called on by the president of the section, preferred deferring his reply till the following day. This morning Dr. Bastian gave an account of his experiments on the contents of hermetically sealed cases of preserved meats, with which the readers of NATURE are already familiar, and reiterated his conclusion that the facts he had elicited were such as to throw on the Biogenists the burden of proof that life did not really, as was apparently the case, originate *de novo* from lifeless materials. Professor Huxley was not able to be present at this discussion, but a somewhat sharp passage of arms took place between Dr. Bastian and Professor Tyndall, each maintaining his well-known view respecting the atmospheric germ theory. The reply of Prof. Tyndall, "Prof. Huxley's lieutenant," as he was described by the president of the section, was not generally accepted as conclusive, in consequence of his apparently not having made himself thoroughly acquainted with the facts of the series of experiments performed by Dr. Bastian.

From Nature, 2, 438, September 29, 1870.

OLD WORLD

SCIENCE RESEARCH COUNCIL

Signs of Stringency

THE annual report of the Science Research Council for the year ending on March 31, 1970 (HMSO, 8s 6d), suggests that the council has now settled down to a steady battle with the British Government on the availability of funds. In a review of the past five years, the council points out that there has in the past few years been a "sharp fall" in the rate at which the budget has been growing. Over the period of five years, the annual budget in real terms has increased by 40 per cent, from just under £35 million in 1965-66 to £46 million in the year just ended. During the same period, however, the university population has also grown rapidly and there is clearly now a danger that the council will be unable to meet the growing needs of university research and its commitments to international projects such as CERN and ELDO.

So far, the council has dealt with the strain on its system by cutting back on capital projects. Because of devaluation and the rapid build-up of ESRO in the mid-sixties, subscriptions to international organizations, which cost £12 million in 1969-70, have accounted for a proportion of the budget which has increased from 18.5 per cent to 25.9 per cent in the past five years. The proportion of the budget spent on research grants has been constant at about 20 per cent, while the policy of linking postgraduate awards to a fixed percentage (16 per cent) of those graduating from British universities in science and technology has made it possible to stabilize the cost of postgraduate awards at 12.1 per cent of the total budget. Over the five years, the most dramatic change in the pattern of the council's spending has been the reduction from 16.2 per cent to 6.6 per cent in the section of the budget spent on research machinery such as the particle accelerator. Although this is in part a consequence of the council's deliberate decision to cut back on spending on nuclear physics (reaffirmed in the report), the council also says that if "the rate of increase of funds available to the council continues to be restricted" there is a danger that capital schemes "required to restore the proper long term balance in the council's expenditure" will have to be postponed. Plainly some of the council's much admired chickens are now coming home to roost, for the policy of selectivity and concentration by means of which attempts are being made to build up centres of research at British universities is creating a need for new and expensive facilities.

As yet, there is no sign that the council doubts the wisdom of its policy of selectivity and concentration. One of the objectives is to select areas of research for special mention when there is a promise of valuable rewards, either intellectual or economic. A second is to choose university departments at which concentrated work is likely to be unusually valuable. One feature of this policy has been the council's decision to give priority to astronomy and to backpedal in its support for nuclear physics. In the current report, the council goes out of its way to reaffirm its belief in the present system for supporting university research in which funds are channelled to university departments by both the research councils and the University Grants Committee. The report says that the Council for Scien-

tific Policy is at present trying to work out a policy for regulating the way in which responsibility for particular research projects should be transferred from the research councils to the UGC—a review which could in the long run lead to a change of policy. In its review of progress in the various branches of science, the council says that the design of the 400 foot radio telescope has been completed and that government approval has been sought, that the northern hemisphere review of optical astronomy is now complete, that demand for time on the Isaac Newton telescope is four times as great as the instrument can accommodate (which makes the case for a northern hemisphere observatory stronger), that discussions with the South African Council for Scientific and Industrial Research about collaboration on astronomy are progressing well, that approval has been given for the building of a 33-metre dish for millimetre wavelength astronomy and that a panel is now at work on the design of a future programme for the Radio and Space Research Station at Slough.

In the past year, the division of the grant giving responsibilities of the Science Research Council into four boards, responsible respectively for astronomy, space and radio, engineering, nuclear physics and science, has been brought into effect. The current report says that 30 per cent of the engineering board's resources is being spent on selected areas of research, including enzyme chemistry and technology, control engineering and polymer science. The engineering board is also hoping to spend up to £500,000 on transport research by 1973–74, chiefly because of the considerable benefits to be expected from university research in this field.

The report has very little to say about the decision of the British Government not to participate in the 300 GeV programme, but it is stated that the nuclear physics board "continues to maintain as its topmost priority the UK's eventual accession" to the project. With the increasing pressure on the council's budget, it seems to be harder than ever to find a way of fitting a contribution to the 300 GeV machine within the straitened budget, but the report says that the nuclear physics board "has managed to achieve a programme that is poised for entry" while permitting "an adequate continuing exploitation" of the domestic laboratories.

The science board congratulates itself on the growth of support for university research in the biological sciences, and says that it will make a special effort to support biological work with industrial potential. One novel feature of the council's work is the way in which it has fanned life into mathematics research and teaching. In the past year, a panel on numerical analysis has begun work, and thus supplements the work of previous panels on differential equations and engineering mathematics. The council is especially pleased with the way in which it has been able to stimulate research by organizing symposia extending for periods of time ranging from a month to a year and organized around groups of visiting fellows attracted to one place by the award of senior visiting fellowships.

INNOVATION

Optimism at NRDC

THE National Research Development Corporation has had a hectic twenty-first year. For the second time

running, the corporation made a small profit on its revenue account, its managing director, Mr John Duckworth, left to take up an appointment with a merchant bank and the green paper on government research which was published in January by Mr Wedgwood Benn, then Minister of Technology, suggested that the NRDC should form part of the proposed British Research Development Corporation. Nevertheless, the NRDC's annual report for 1969–70 shows that the growth of business noted during the previous two years has been maintained or even bettered.

The financial targets at which NRDC was aimed in 1949 are still a long way off, however. It was originally hoped that the corporation would back innovations which would bring in enough revenue to finance its continuous development work. During the previous government's reign, the notion that NRDC might become a way of channelling funds to industries, without immediate return, became more fashionable. The annual report shows that the gap which has accumulated over the past 21 years between NRDC's development expenditure and its income stands at some £2.7 million. But the financial prospects look bright enough for the report to suggest that "the overall picture is not unsatisfactory considering the high risk element in the business underwritten". One of the questions now to be decided is whether the new government will be satisfied by these promises.

The corporation last year invested more money than ever before—£5.6 million compared with £3.6 million in 1968–69—and happily last year's income was a record £4.2 million, much of which was in foreign currency. This allowed the corporation to make a small profit on its revenue account for the second year running. Much the biggest money spinner on NRDC's books is the antibiotic cephalosporin, originally developed in Oxford and brought on the market in 1964. Last year, the drug brought in about £2 million, or nearly half the corporation's total income, and the NRDC is evidently hoping that its investments in hovercraft variations, including the tracked hovercraft, carbon fibres and microelectronics, which comprise most of its present work, will eventually provide a substantial income.

Since the corporation was established, it has received nearly 23,000 submissions for support, from which it has chosen 5,076 projects. Last year, 105 new projects were started, bringing the total number of current projects up to 377, and the corporation now believes that there is a wider recognition of its activities—one of its chief grumbles in the past has been that industry is insufficiently aware of what NRDC does. The projects backed last year also highlight the corporation's new policy of supporting a firm's own projects on a joint venture basis—in effect, sharing the risk capital. In its early years, NRDC tended to back projects from universities and government laboratories, but the report shows that 162 of the 377 current projects are joint projects with industry.

A ticklish subject which the report leaves well alone is what will happen to the NRDC in the future. The green paper saw the corporation as part of the proposed British Research Development Corporation, but a firm statement of the new government's views on government research establishments—and indeed on the future Ministry of Technology, NRDC's master—is unlikely to be made until the autumn.

PARTY CONFERENCES

Back Seats Again

THE season for political party conferences has come round again, but signs of new thinking from the grass roots of the three main political parties are unlikely to find their way to the conference floor—it is too soon after the general election for that. And, by the same token, issues of science policy are notably few and far between on the conference agendas.

The chief item of interest, in the circumstances, is the appearance of Mr John Davies, the new Minister of Technology, in a debate on government and industry. The debate is due to take place at the Conservative Party Conference in Blackpool on October 8, and it will centre on a motion by the South Nottingham Conservative Association "calling for a stronger role for private business in the national economy". Mr Davies's reply to the debate should give him his first opportunity to outline his thinking on the role of the Ministry of Technology in industry, and on the green paper on government research, published by the Labour government. The government is already pledged to reduce government intervention in industry, and it will be no surprise to see Mr Davies preside over the demise of a ministry which has always been something of a *bête noire* to the Conservative party.

On education, Mrs M. E. Thatcher will find herself replying to a motion in which she is congratulated on withdrawing the compulsion for local education authorities to submit schemes for comprehensive secondary education. But it will be interesting to see whether she has yet been able to coordinate her policy on the expansion of higher education.

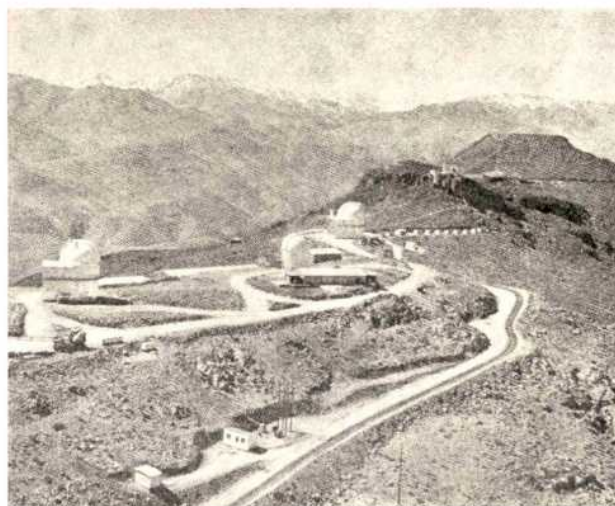
While the Conservative Party Conference promises to be something of a showpiece, in which new ministers are presented to the rest of the party, the Liberals and the Labour party will be more concerned with analysing what went wrong in the election campaign. But there is every sign, however, that science policy will not take a back seat in politics for too long—the popular concern over pollution has found its way into a motion to be debated at the Conservative conference, and a motion submitted to the Labour conference by Nottingham Central Labour Party includes a comprehensive list of suggestions for pollution control. The debate at the Conservative conference will give Mr Peter Walker a chance to say what he plans to do.

ASTRONOMY

CERN lends a Hand

AN agreement was signed in Geneva last week between the European Organization for Nuclear Research (CERN) and the European Southern Observatory (ESO) for collaboration in the design and construction of a 3.6 m conventional telescope, third in size only to that at Palomar, California, and that under construction in the Crimea. The telescope, costing about £8 million, will become the chief research instrument of the ESO and will be situated at the site of the existing observatory at La Silla in Chile, where it is expected to start operating by 1976.

A new division is to be set up at CERN comprised of 30 to 40 astronomers and engineers working under the head of the telescope division of ESO, Dr S. Laustsen.



The ESO observatory at La Silla, Chile. The new telescope will be situated on the mound in the top right of the picture.

Although the division will be autonomous with its own budget the idea is that it should share in the support facilities of CERN and draw on the experience of the 16 year old nuclear laboratory in the design and construction of large installations.

The three existing telescopes at the ESO in Chile, which have been operating since 1967, are manned by a permanent staff of about ten astronomers together with a regular complement of visiting astronomers from the six member states of ESO, namely Belgium, Denmark, France, West Germany, the Netherlands and Sweden. All these countries are also members of CERN. A 1 m Schmidt telescope is also being installed at the ESO, La Silla, comparable with the Palomar Schmidt used for sky survey work, and is expected to be in operation by the middle of 1971.

The site in Chile, selected by both the ESO and the American Southern Observatory, was chosen originally for the favourable weather conditions and because ESO astronomers were particularly interested in celestial objects visible only in the southern hemisphere. The link-up with CERN may be seen as giving substance to the view that a better understanding of high energy physics will as likely as not come from astronomy.

COMPUTERS

Green Light for New Giant

THE merger between the computing interests of Honeywell and General Electric (USA), which has just been given the green light, produces an international computer giant second only to IBM. The combine expects to reach a 10 per cent share of the world wide computer market compared with 70 per cent for IBM. General Electric is represented in the United Kingdom by its subsidiary, GEIS Ltd, which has considerable experience in time-sharing and information services but no manufacturing capabilities in its own right. Honeywell Ltd is the much larger subsidiary, with a plant at Newhouse in Scotland, and it is strong in small and medium scale computers. The new company Honeywell Information Services Ltd, formed by the amalgamation of the two subsidiaries, will add the large scale systems made by

General Electric in America to its catalogue, giving a broad base of supply.

Honeywell is a relative newcomer to Britain, but has been expanding at roughly 40 per cent a year. It is, however, weak on services, having only just opened its first time-sharing bureau in Slough. The merger will bring to Honeywell considerable software knowhow. The sales side of the combine will be structured to form two divisions: an information services division handling time-sharing, commercial education, contract programming and other bureau activities, and an equipment division selling all ranges of hardware and data entry devices, and sticking to the all-in or "bundled" pricing policy.

In Britain the disparity in size and function of the two subsidiaries will simplify the creation of Honeywell Information Systems Ltd. Sister companies in Europe will have rather more difficulty in unifying. Honeywell and General Electric are nearly the same size in some countries and are competing strongly with one another, particularly in West Germany. The impact of their eventual merger will be correspondingly greater than in Britain. One can expect some confusion initially by analogy with the English Electric-ICT merger which is even now bedevilled by the problems of incompatibility of the two systems. In fact, incompatibility within Honeywell-GE systems may lose some of Honeywell's new-found customers. Mr G. Bosnan, managing director of HIS Ltd, feels, however, that it will not take too long to develop a wide range of compatibility or peripheral devices, and he believes that "the software compatibility problem is eased to the extent that higher level languages are used".

The new combine, the third biggest computer concern in Britain, will provide some competition for ICL. Its share of the market will go up to about 10 per cent, well behind ICL and IBM with about 40 per cent each. It is expected to become particularly important in the commercial software services field for which there is an obvious need. It will also be competitive with IBM, and may even assist in reducing the IBM dominance of the international computer market. Yet the broad range of supplies and services offered by the new company makes it vulnerable. It could be too thinly spread for its size, requiring impossible investments over a wide area, and it may be unable to offer the best specialist services which could be provided by smaller concerns.

SPACE RESEARCH

Skylark Rejuvenated

by a Correspondent

BRITAIN'S space experiment work-horse, the Skylark sounding rocket, has achieved a new lease of life through the development of a low-cost, packageable launcher system. Curiously this has been developed by West German interests, forced into exceptional solutions by having no suitable rocket range on their own territory. Essentially, the system comprises a single rail guide, mounted on four legs set 90° apart supported by a concrete pad. Skylark is launched from the rail itself which has a range in elevation of minus 5° to plus 100°.

The system has proved substantially cheaper than the permanent installations and it is also more flexible. It is claimed to be able to launch sounding rockets not exclusively limited to Skylark for research programmes



Skylark being launched from the MAN launcher at the Andoya range, Norway.

"at any geographical location with minimum site preparation". It is fully compatible with Britain's other two chief research rockets, the smaller Petrel and Skua, which are fired from tubes and can also be mounted on the adjustably pointed rail.

The British Aircraft Corporation, makers of the Skylark, have recently entered into an agreement with DFVLR of Munich, sponsors of the launcher developed by MAN, to market Skylark using this launch system. The new export sales scheme promoted by BAC also includes the other British sounding rockets which are made by Bristol Aerojet.

ENGINEERING

Fluids at the Fore

from our Special Correspondent

East Kilbride, Scotland

HOPES are again alive at the National Engineering Laboratory here for the use of hydrostatic transmission in ordinary motor vehicles. Trials with a BMC Mini seem to have confirmed that a fluid drive to all four wheels gives quieter and more efficient performance, although reliability and cost are still in doubt. Visitors to the open days last week may have caught a glimpse of a hydrostatically driven Mini leaving a conventional Mini stranded at the post on a stiff climb up a greasy road surface.

One of the newer services which the NEL is offering to industry is what is called the Metrology Club of Scotland, through which Scottish companies may use the £300,000 worth of precision measuring equipment at the NEL for a fee of £150 a year. The NEL runs a wide range of test facilities for industry, among which is laboratory simulation of road conditions for testing

new styles of chassis. An aluminium lorry frame is now being subjected to vibrations calculated to be those from a typical minor road in Britain, and it is hoped to weed out design faults (or design excesses) without extensive road trials.

Although the focus of attention for carbon fibres has been in aircraft applications, the NEL is keen to explore the potential of fibre composites for building simple structures. One outlet for this is in sports equipment, for objects such as golf clubs and squash rackets, and an experiment has shown how the peculiar distortions suffered by a squash racket can induce unusual fractures in a fibre reinforced object. Another fibre reinforced device pioneered at the NEL is a measuring frame which may be mounted on a lathe so as to carry a probe for tracking the diameter of a large roll. This is one of a range of light, stiff measuring tools being developed.

An attempt is also being made to coax smaller firms into using computer aided design. The NEL has started a regional experiment in computer aided design in which participants may use the NEL Univac 1108 computer free of charge for a limited time in return for contributing to the program library and providing their own on line equipment. A scheme is also under way to teach computerized production control at the new Birniehill Institute, a training centre adjoining the NEL and associated with Strathclyde University, in which postgraduate students and people from industry may study engineering design and the control of advanced machine tools. Several new concepts are being taught in group technology—in which a whole family of components are manufactured together—and in workshop scheduling. A bureau service is being provided for a computer program in workshop analysis and scheduling from the Atomic Energy Research Establishment, Harwell.

TERATOLOGY

Another New Society

A GROUP of scientists with a common interest in congenital malformations has formed the European Teratology Society (ETS), which begins its formal existence on October 1. Although some people might say there are too many societies already, the founders of the ETS are convinced that there is an urgent need for such an organization in Europe. The problem is that people working in teratology are scattered throughout the different disciplines of universities and in hospitals, government institutes and the pharmaceutical industry. They badly need a forum for discussion and self-criticism, according to Dr J. B. Lloyd of the University of Cardiff, one of the founders of the new society.

The decision to form the ETS was taken a year ago during the international conference on congenital malformations at the Hague, and enquiries made since then have brought in more than four hundred favourable responses. Since January this year, a literature search has been in progress, on behalf of the society, at the Karolinska Institute in Stockholm. This is known as *Teratology Lookout* and at present has about five hundred people on its mailing list.

Stockholm is to be the home of the ETS, and its acting secretary is Dr K. S. Larsson who works in the Department of Anatomy at the Karolinska Institute.

There is to be no new journal to go with the new society; members are to be advised to support *Teratology*, the journal of the Teratology Society, which is the American cousin of the ETS.

As a first move in this direction the abstracts and free communications of the first meeting of the ETS will be published in *Teratology*. The meeting is to be held at Cardiff from April 14 to 16 next year, and will include three symposia on topics which are intended to indicate the scope of interests embraced by the society. A distinguished group of teratologists, including the president of the ETS, Professor H. Tuchmann-Duplessis of the Faculty of Medicine in Paris, will give contributions on the aetiology of human birth defects; the problems involved in screening drugs for potential teratogenic properties, and the management of spina bifida. Further information about the meeting can be obtained from Dr J. B. Lloyd, Department of Biochemistry, University College, Cardiff.

EDUCATION

Nuffield on Trial

from a Correspondent

A PREVIEW of the sixth form course in physics, to be published by the Nuffield Advanced Physics Project early in 1972, was provided in Birmingham last week. The occasion was a conference held by the education group of the Institute of Physics and the Physical Society. The principal purpose of the meeting was to discuss the implications of the new course for higher education, and about seventy institutions, universities, technical colleges and colleges of education were represented.

The organizers of the project, Dr P. J. Black (University of Birmingham) and Mr J. M. Odgborn (Chelsea College of Science and Technology), explained that they had selected for the course a set of topics which could be inter-related so as to do justice to the principal themes and types of thinking in physics. The course is now being taught in eighty-five schools and two technical colleges, and teachers from five schools described their experiences. Their principal message was summed up by Mr A. L. Parker (Banbury School) who said that his role had been changed so that he saw himself as a leader of a group who, with careful guidance and a great deal of support material, were discovering physics. Miss B. M. Jennison (Godolphin and Latymer School) commented on the emphasis in the course on promoting discussion among students: patience and persistence were needed in the face of initial diffidence or resistance, in order to build up habits of questioning and discussion in the classroom.

The change in attitude and approach to science which the course seemed to be promoting was stressed by three speakers from universities, who were attempting to assess its implications. Dr B. J. Brinkworth (University College, Cardiff) had several criticisms, but felt that the strengths of the course—its structure, the emphasis on a few important themes, the use of experimental work, the use of order of magnitude calculations and the attention devoted to models and concepts—augured well for the quality of future students.

Professor G. Carter (University of Salford) was more pessimistic, feeling that, although school science

teaching would be improved greatly by courses of this type, universities would change only slowly and would for a long time offer courses which might inhibit rather than extend the skills and attitudes developed in schools. If the progress in schools could be matched in universities, they might produce graduates whose habits of active and independent enquiry would cause difficulties in industry where the traditional structures often left little scope for young employees with such attitudes.

When implications for first year physics and engineering courses in universities were discussed, several speakers agreed that a change in attitude to the teaching of science and engineering would be needed. But it was clear that any policy to encourage students in higher education to learn in a new way by different patterns of teaching might face severe difficulties because of constraints on staffing, timing and space. New developments in university teaching, however, were quoted and if the response of participants at the conference is a reliable guide, then higher education will adapt to take advantage of changes if detailed information about these changes can be presented in good time to a sufficiently large number of teachers.

PESTS

Rats March On

THE four year campaign by the Ministry of Agriculture to contain the spread of rats resistant to Warfarin has recently been called off. For the time being, rats will have to be controlled by conventional rodenticides. These poisons—arsenic, zinc phosphide or fluoroacetamide—are acute and they work on a single dose, provided that the rat ingests a sufficient amount. There is, however, no means of ensuring that household pets and other animals are safe from accidental poisoning. The skill of the operator is what matters.

Warfarin is by far the most efficient and safest way to destroy rats. It is slow to exert its toxic effect so that several doses are needed, thus reducing the risk of accidental poisoning. Moreover, the rats are then unable to associate their illness with the food source, and do not learn to avoid the Warfarin bait. The material is an anticoagulant, which functions by interfering with the ability of the blood to clot. Chemically, Warfarin has a structure similar to that of vitamin K, and one of its effects is to block the synthesis of clotting factors in the liver. The action of vitamin K is also blocked by Warfarin, and in fact rats resistant to Warfarin have an exaggerated vitamin K requirement.

Anticoagulants were in worldwide use, with much success, until outbreaks of resistance were discovered in 1960 in Scotland, Denmark and Wales. The continued use of Warfarin in rat control killed the sensitive rats, giving the resistant rats an opportunity to spread. Apparently the resistance is controlled by a single dominant gene, which means that resistance occurs in most of the offspring of resistant parents. The artificial selection operated by humans, in the continued use of Warfarin, greatly assists the resistant rats to spread. The resistant rats are at a disadvantage because of their large requirement for vitamin K. They would probably gradually die out if Warfarin ceased to be used.

The Ministry of Agriculture attempted to halt the

march of the resistant rats by throwing a cordon around the affected areas. The three-mile-wide ring in Montgomeryshire and Shropshire was meant as a holding action to give the laboratories sufficient time to develop an entirely safe rodenticide. Landowners were warned to use only conventional rodenticides within the ring. The rats were spreading at 3 miles a year, but the cordon seems to have checked them until fairly recently. The scheme failed because some landowners continued to use Warfarin within the ring, thus undermining the ministry's effort. In addition, the outbreak of foot and mouth disease reduced the mobility of the ministry team.

The fact that resistance is easily transferred and that rats tend to migrate in search of food means that resistance will be appearing in the cities before long, provided the resistant rats can get hold of sufficient greenstuff to satisfy their vitamin K requirement. The ministry is testing new drugs as a matter of high priority to avoid the unpleasant prospect of the use of highly toxic conventional poisons in cities and towns. Norbormide is one possibility although it is not as safe as Warfarin. Two other unspecified drugs are undergoing field testing by the ministry at present.

FOUNDATIONS

From 'Q' and 'F' to Nuffield

PROFESSOR Clifford Butler, professor of physics at Imperial College, London, has been named as the next director of the Nuffield Foundation. He will take over on January 1, 1971, to fill the position left by Mr Brian Young, who takes up his appointment next month as Director-general of the Independent Television Authority.



Professor C. Butler.

Professor Butler will take over the directorship of an organization which distributes more than £1 million in research grants every year, but which is perhaps best known for its sponsorship of schools teaching projects. The new director of the foundation is certainly no stranger to this type of work—he was joint chairman of the committee which came up with the controversial proposals for 'Q' and 'F' level examinations in the sixth form, and he is a member of the Standing Committee on University Entrance.

Programme for an International Centre

A MEETING at the Royal Society in London which finished earlier this week seems to have laid the foundations for what may be a unique international research centre—the International Centre of Insect Physiology and Ecology at Nairobi. The scheme, which has been hatching for the past two years, will eventually take the form of a research establishment on the campus of University College, Nairobi, but under the management of a consortium of scientific academies from Europe and the United States as well as from East Africa.

Although it will be some time before the future of the centre is financially assured, the first research fellows will begin work in the next few days, using for the time being rooms provided by scientific departments at the university. The governing body, which will by now have met in Nairobi, is also hoping to be able this week to authorize the expenditure of \$10,000 provided by two Dutch universities for the construction of a temporary research building on the site earmarked for the construction of the main centre. It is hoped that before the end of the year, three or four research fellows will be at work under the direction of Professor Thomas Odhiambo, professor of insect physiology at University College, Nairobi.

Unlike many other research establishments, established over the years in Africa, the International Centre of Insect Physiology and Ecology is not primarily aimed at problems of local significance but, rather, at matters of long term scientific benefit. Professor Carroll L. Wilson, the chairman of the governing board of the international centre, explained earlier this week in London that the scientific interests of the people chiefly concerned with the research programme of the institute were by good chance of a kind that might yield practical benefits to the region in which the studies would be carried out, but that the immediate reason for siting the centre at Nairobi was the happy coincidence of opportunities for research on tropical insects and the enthusiasm of the university, and particularly of Professor Odhiambo, for this project. In short, valuable though the proposed centre may be as an illustration of how national academies of science may collaborate with each other on work of value to developing nations, the Nairobi centre would not easily be replicated elsewhere.

To begin with, the research programme includes the study of insect hormones which control growth and reproduction, particularly in tsetse flies and locusts. There is also a plan for providing facilities for the study of pheromones, insect behaviour, mosquito genetics and the chemistry of the plant products which can function as insect hormones. Although the breadth of this research programme has been determined by the need somehow to bring together the kind of interdisciplinary interests likely in the long run to yield practical as well as intellectual benefits, it is also recognized that much of the momentum for the development of the centre will be provided by the interests of those scientists who are involved with the centre either as members of the governing board, as directors of research responsible to the director but not necessarily living at Nairobi and by the members of

the advisory council who are for practical purposes delegates of national academies of science.

As research projects of developing countries go, the Nairobi centre is an ambitious undertaking. In the next five years, it is hoped to be able to spend £474,000 on capital equipment, and that recurrent expenditure will amount to £312,000 a year by the end of this period. Although the full time staff of the institute at present allowed for in the budget amounts to fewer than a dozen paid research fellows, it is intended that the centre should be at the hub of a network of research in insect physiology involving not merely the laboratories at Nairobi but also those which have become centres of excellence in the nations with national academies contributing to the management of the centre. One particular feature of the scheme is that for the appointment of African scientists to fellowships allowing them to carry out research at the postdoctoral level in the laboratories of scientists associated with the centre, usually as directors of research, on the understanding that after a spell abroad, they will return to Africa. In this way, it is hoped that the centre will demonstrate its intention to be of cultural and educational value to the region as well as—possibly in the long run—of practical help with the solution of African problems as well.

The origins of the project are interesting in themselves. The first stimulus seems to have been a paper presented by Dr Carl Djerassi to the Pugwash Conference in Sweden in September 1967 and later summarized in the *Bulletin of Atomic Scientists* (January 1968, p. 22). Djerassi argued that the setting up of centres for basic research recognized internationally to be of high quality would benefit developing countries by demonstrating that scientific development does not have to recapitulate the pattern of change observed in developed countries, by providing help with practical problems and by providing opportunities for educational advance in the region as well as serving as means of keeping good scientists at home. By all accounts, one of the first proposals was that Nairobi should become an international centre but that it should be chiefly concerned with oceanography. Professor Odhiambo seems to have been the first to argue that Nairobi was especially suited to a study of insect physiology and ecology. In the past two years, the project has been vigorously supported by the National Academy of Sciences in the United States and by the American Academy of Arts and Sciences.

At the international committee meeting in London this week, the governing board emphasized that the project would not be thrown open to all potential members but only to those academies which are likely to have an important scientific contribution to make to the work of the Nairobi centre. The speed with which all these plans can be brought to fruition will depend very much on the response of the United Nations Development Fund to an application for funds which is to be made and on the willingness of the charitable foundations to help. In the meantime, it is clear that there is enough steam in the project for it to be able to get under way with *ad hoc* gifts of equipment, research facilities and bits and pieces of money.

NEW WORLD

Continuing Debate about Backdoor Science

by our Washington Correspondent

SELDOM since the early church was driven to schism over the word *filioque* in the creed can so massive an establishment have been racked by so little a text as American science is being racked by the Mansfield amendment. A 40-word sentence tacked onto last year's military procurement act, the amendment merely enjoins that the research conducted by the Department of Defense bear a "direct and apparent relationship" to specific military need. Although the clause is now approaching its first anniversary, debate about its real and imagined consequences continues to smoulder. This month Mr Emilio Q. Daddario, chairman of the House subcommittee on science, research and development, called on President Nixon to set up a government conference to study the impact of the amendment on federal research. A few weeks ago the author of the amendment himself, Senator Mike Mansfield, offered a vigorous justification of his intentions in a written statement to Mr Daddario's committee. The Senate, at least, is still persuaded of the justice of Mr Mansfield's arguments, since last month it rewrote the amendment into this year's version of the military procurement authorization bill.

The logic of Mr Mansfield's amendment is that basic research, and the universities where it is conducted, should largely depend on the National Science Foundation for support, not the Pentagon. The case against the amendment, simply stated, is that it is a directive to switch horses in mid stream before the new horse has hove in sight. Mr Mansfield has admitted the peril of the manoeuvre in as far as he acknowledges that "to get out of comfortable, well worn ruts sometimes requires heroic measures". In his written statement to the Daddario committee the Senator lays the blame for the situation on the indolent folly of the scientific community in allowing the Pentagon to usurp the proper functions of the National Science Foundation. He also blames the Office of Science and Technology for countenancing this development and looks to Congress to remedy its long neglect of science policy and clear up the mess that has arisen.

Mr Mansfield's argument is that over the past 25 years the scientific community came to rely on the Department of Defense because its hand-outs, being less subject to Congressional scrutiny, were more readily available than those from civilian agencies. Universities, although unwilling to accept direct subsidies for fear of losing their independence, acquiesced in Pentagon research grants which in effect helped to defray a good part of their overheads. "For

years Defense funding provided a very stable source of research money. It was the easiest path for the research community to follow."

The culmination of this primrose path was that the Pentagon, with the tacit collusion of the academic community, succeeded in establishing within itself a kind of "backdoor National Science Foundation" which has run the gamut in its support of research projects "from the most esoteric examinations of ornithology to the study of broad social movements in foreign countries". Mr Mansfield found his worst suspicions confirmed when Dr John S. Foster, Director of Defense Research and Engineering, made it clear during Senate hearings in 1968 that "the Pentagon believed all fields of science and technology were open to it, that it saw no inconsistency in funding basic research in fields already funded by civil agencies, and that all research projects it sponsored were somehow relevant to defense needs".

The amendment which Senator Mansfield subsequently introduced "is neither anti-military nor anti-research". Rather it is a mandate to reduce the research community's dependence on the Department of Defense and to bolster the National Science Foundation which "since its creation has been the orphan child of the federal government's science policy". Mr Mansfield remarks that since 1955 the NSF has been given only \$2,000 million compared with the \$3,000 million spent by the Pentagon on basic research.

What have been the consequences of the amendment? Mr Mansfield reports that much progress has been made despite the resistance that has lingered in some quarters. For a start, the NSF has been authorized to receive for the present financial year an increase of \$75 million over its last year's stipend, while the Pentagon's share of basic research funds will be \$50 million less than that of the NSF. But the Senator criticizes the manner in which the National Academy of Sciences refused the Pentagon's request to help decide on the military relevance of the research projects it was supporting. The president, Dr Philip Handler, volunteered the Academy's services in a follow-up review and agreed that it would be useful for the Academy both to formulate principles by which the carrying out of the amendment might be guided and to attempt to forecast the consequences of the amendment for government research policy and the national welfare. Mr Mansfield comments that "while I did not ask the Academy to do so then, experience with Section 203 [as the amendment is technically known] indicates that it should do so now".

NATIONAL R & D INVESTMENT, AS ESTIMATED BY THE DEPARTMENT OF DEFENSE

	(In 1000 millions of 1966 dollars)				
	1955	1960	1965	1968	1970 (est.)
US	5.1	13.7	20.6	25.4	24.6
USSR	3.5	7.8	13.9	17.7	21.3

The tangible effect of the amendment on the Pentagon's support of basic research has fallen short of calamitous. Of the 6,600 projects reviewed, only 220, involving funds totalling \$8.8 million, were judged to fall within the amendment's purview, a sum that represented less than 1 per cent of the \$1,295 million of federal funds devoted to university research and development in the last financial year. At the same time the Department of Defense received a cut in its research budget for 1970 of \$64 million, notwithstanding Section 203.

Mr Mansfield concludes his statement to the Daddario committee by remarking that "all that is required under Section 203 is relevance, which is not a dirty word as some critics of the section sometimes seem to suggest". Unfortunately the budget for the present financial year does not indicate that the Administration has taken the unique opportunity of redressing the imbalances in federal research funding that was afforded by the amendment. "To carry out the intent of Section 203 will require new ventures in interagency coordination. That is the responsibility of the reorganized Bureau of the Budget and the Office of Science and Technology under the President. Thus far, unfortunately, the White House science office apparently sustains the rigid opposition of the agencies to Section 203. That is most unfortunate because if there are to be improvements in coordination and a shift in the emphasis of federal policy with regard to the support of basic research, it is going to take a joint effort by the President and the Congress. . . . I hope we can look to the scientific community for advice. Yet I recall that this community speaking through the National Academy of Sciences in 1965 was unable to answer the questions on how much money should be spent for research and how it should be divided up."

The Senate has accepted the burden of the Majority Leader's argument, as indicated by its action late last month in adding the amendment onto the military procurement authorization bill for the present financial year. The amendment was not included in the version of the bill passed by the House of Representatives and its appearance in the final act will depend on whatever bargain is struck between House and Senate. However, the Senate seems to have been less than wholly content with the way the Mansfield amendment has worked out in practice since by a vote of 68 to nil it approved a second amendment expressing the "sense of the Congress" that basic scientific research should receive a greater share of government support. The amendment, proposed by the chairman of the Senate Armed Services Committee's subcommittee on research and development, Mr Thomas J. McIntyre, also calls for an increase of 20 per cent, or some \$100 million, in the National Science Foundation's budget for next year.

The Senate may concur with the philosophy of the Mansfield amendment but the Department of Defense seems still to adhere to the view that where basic research is concerned, the world is its oyster. Last week, in the last of its hearings on whether the United States should have a national science policy, the Dad-

dario subcommittee heard evidence from the Director of Defense Research and Engineering, Dr Foster. The size of the Pentagon's research effort, Dr Foster explained, is determined by the level of activity of the USSR and other communist countries. The individual services have been very reluctant to drop any of their basic research for fear that the Soviet scientists might come up with a surprise. "We must do that R & D which needs to be done." This blatant tautology, which seems to be the cornerstone of the Pentagon's defence against the Mansfield philosophy, prompted a member of the subcommittee to observe that it sets no limits on the scope of military research. The reflexion programmed Dr Foster back on the other loop of his argument, the size of the Soviet threat. According to Pentagon estimates, the Soviet national investment in R & D has been increasing so fast (see Table) that it will surpass that of the United States within the next few years, while in the area of R & D related to defence, the USSR has already surpassed the USA, being expected to spend some \$16 to \$17 thousand million this year compared with a combined total of £13 to \$14 thousand million for the Pentagon, the Atomic Energy Commission and NASA. How did the Pentagon arrive at these estimates of the Soviet Union's research investment? That was a terribly complicated fiscal question, Dr Foster explained, but it depended, by and large, first on estimating the "effectiveness" of a rouble and seeing how many would be needed to accomplish the military programmes the Soviets had under way and, second, on estimating the cost in dollars of reduplicating the Soviet military research effort. The two methods agreed with each other to within 10 per cent, Dr Foster said.

On the relationship between the Department of Defense and the National Science Foundation, Dr Foster made clear his antipathy to the idea that defence research should be transferred from the former to the latter, chiefly because the introduction of a third party into the process of coupling research to development would lead to great inefficiencies. Equally it would be a serious threat to national security if the Pentagon's support of university research were to be significantly curtailed since without such a connexion it would be cut off from the advice of university scientists experienced in defence matters.

With the positions of the Pentagon and the Senate so far apart, it is little wonder that Mr Daddario has seen the need for some kind of arbitration on the consequences and significance of the Mansfield amendment. In a letter sent to President Nixon on September 8 he proposes that an executive-legislative conference on federal research should be convened so as to resolve the "confusion, uncertainty and anxiety" that have resulted from Section 203. The conference would include the heads of the departments and agencies that are the major sponsors of research, delegates from the relevant committees of the House and Senate and representatives from the White House. It remains to be seen whether the Administration will pick up this gauntlet and, if it does, how far the conference will be led from the immediate topic of the Mansfield amendment to a discussion of the whole field of national science policy. This is, perhaps, what Mr Daddario may be hoping for; the hearings conducted by his committee over the past three months have prepared the way for just such an outcome.

REINCARNATION

Karma Revealed, or Nearly

from our New York Correspondent

CAN an English girl find true happiness as an Indian in her next life? Dr Jamuna Prasad, who has been investigating this and five other cases of reincarnation, believes she cannot. In a talk to the American Society for Psychical Research in New York last week, Dr Prasad presented the findings of a study on how the earlier personality determines the personality of the reincarnation.

Dr Prasad, who is deputy director of education, Allahabad, Uttar Pradesh, India, and head of a parapsychology unit there, believes he has found that newly reborn children between the ages of two and five show strong memories of their previous life. When these memories are in strong disagreement with their present way of life—because of pressure from their new parents and their present socio-economic situation—conflicts emerge.

Is it only "the residues of personal experiences" or the entire personality that carry over into the new incarnation? To study this problem, Dr Prasad sent out teams to conduct personality trait questionnaires for both the past and the present incarnations. To control the study, a team of impartial judges was asked to match the past and present personalities. Their success rate was quite high, but since there were only six pairs of subjects, two of whom were girls, Dr Prasad admitted that it would not be difficult to match them by elimination.

In general, the degree of similarity between the two members of each pair was quite strong, except in one case where the first incarnation had such a passion for a certain type of gruel that he finally died of overeating; his reincarnation, naturally enough, could not stand the sight of it.

Unfortunately, since these tests were not carried out under the most rigorous and ideal conditions, the results are still open to some doubt. In the first place, in all but one case the two parts of each pair live or lived near each other so that the reincarnation was discovered first by the two families and their friends, who then called in Dr Prasad. The only exception was the case of the Indian girl who had lived in England in her previous life, and she was unable to recall enough specific details to enable the investigators to identify her previous incarnation.

Secondly, the first incarnations not being extant, their personality questionnaires had to be filled in by their relatives who, already having decided that their kinsman had been reincarnated, would tend to relate his character to that of his new incarnation. And thirdly, since specific likes and dislikes are often controlled by the social and physical environment and since all but two of the pairs came from similar backgrounds within the same area, it would be difficult to say on the basis of these tests alone that the similarities were due to reincarnation. Significantly the Indian girl who had previously lived in England could not adjust to her present life—she preferred meat to the diet of her present vegetarian family and missed eating with a knife and fork. Another boy who had previously been a Brahmin was reincarnated into a

lowly caste and was very unhappy, refusing to eat any food cooked by his new family.

Dr Prasad is anxious to carry out more extensive work, with more cases and follow-ups through adulthood. "There is a great need to enlarge the number of cases and improve the tools, for if we can understand these seemingly impossible cases, they may completely change our philosophy and our outlook on life."

Miscellaneous Intelligence

DESPITE the unfashionability of its trade, the Pentagon has apparently had no difficulty in recruiting all the scientists and engineers it needs this year. Indeed, the quality of scientific manpower has been improving, if degrees are anything to go by. More than 10 per cent of the scientists and engineers now employed by the Department of Defense hold PhDs, compared with 8 per cent two years ago.

"CLOAKED with dull invisibility, the House of Representatives has plodded along its disastrous course, largely unaffected by citizen outrage." So says Environmental Action, a movement which has drawn up a list of the twelve Congressmen whose defeat in the approaching elections would best improve the environment. To qualify for this list a Congressman must, among other tests, have demonstrated a singularly bad voting record on environmental matters. Curiously, no less than four of the "dirty dozen" are Republicans from Indiana.

EARTHQUAKES and the San Andreas fault permitting, the 180th meeting of the American Chemical Society will be held in San Francisco on August 24 to 29, 1980. The terrible compulsion that makes it necessary to plan the dates and sites of the society's six monthly jamborees a whole decade in advance is the Gargantuan size, beyond all modesty and proportion, to which these events have swelled. Admittedly only 8,500 delegates attended the meeting that was held at Chicago last week, compared with the usual turn-out of 12,000 to 14,000, possibly because travel grants are in shorter supply this year. But the delegates could listen to any of 2,049 papers, embodying the labours of some 4,000 individual authors. Doubtless the sum total of human knowledge is considerably added to on these occasions but the verbal pollution engendered by 2,000 papers every six months seems somehow excessive.

"EVEN though the rise of science might have something of the impact of a 'revelation' in sociological terms—that is, as a creation of evolutionary potential which is realized as the years go by—it is still a revelation which is very firmly embedded in human society and must be visualized as a phenomenon taking place, as far as we know, wholly within human society. We have to regard science as a 'phylum' (as the term is used by Pierre Teilhard de Chardin), that is, as an expanding movement within the four-dimensional space-time continuum of the social system." From an article in the *Bulletin of Atomic Scientists* by Kenneth W. Boulding of the Institute of Behavioural Science at the University of Colorado.

NEWS AND VIEWS

Unwinding the Double Helix

EVER since Watson and Crick proposed in 1953 that the structure of DNA is a double helix of two strands held together by hydrogen bonds, molecular biologists have cheerfully assumed that DNA replication takes place by the unwinding of the strands, each of which then assembles a new daughter strand by complementary base pairing. The experiment by which in 1958 Meselson and Stahl showed that DNA replicates semi-conservatively has often been misinterpreted as support for this model, but that experiment actually showed that each of the two daughter duplexes contains one of the two single strands of the parental double helix and one newly synthesized strand. By itself, that is no proof of the mechanism involved in the production of the strands.

So why has it been accepted as divine truth that DNA unwinds for replication? Why has it been assumed to be eccentric or even heretical to suggest that the double helix itself may be used as a template for formation of a quadruple helix, which would only then split into double helices? Analogous proposals that DNA might function in the transcription of RNA without unwinding have, by and large, been viewed as equally implausible. The fact is that unwinding of the parent duplex is by far the simplest and most elegant way to explain semi-conservative replication. The use of complementary base pairing provides such a ready way to account for the specificity of replication that it would be a surprise were the mechanism to be otherwise. This remains true even allowing that the replicating enzyme itself plays an important part in maintaining the accuracy of replication (see *Nature*, **221**, 1128; 1969). Much the same sort of arguments support the idea that DNA unwinds, although in a more limited way, to act as template for the synthesis of RNA.

But if DNA unwinds in replication and in transcription, what mechanism is responsible? DNA double helices are stable structures which are unlikely just to fall apart in the cell. Do the enzymes which replicate and transcribe DNA also unwind it? Or is some separate enzyme action involved? So far as transcription is concerned, all the experimental evidence is consistent with the proposal that the sigma subunit of RNA polymerase, which helps to initiate RNA synthesis, may do so by "melting" a small region of DNA into its single strands (see *Nature*, **226**, 1093; 1970); as the remaining RNA polymerase core enzyme progresses along the DNA, it continues to synthesize RNA in a region of local unwinding, which is transient because the DNA duplex reforms behind the enzyme as the DNA strand displaces the newly synthesized RNA interloper.

The belief that DNA unwinds in replication is vindicated by experimental support for the notion

that unwinding is catalysed by a specific protein and is too important to be left to the vagaries of chemical action. On page 1313 of this issue of *Nature*, Alberts and Frey show that *Escherichia coli* cells infected with phage T4 contain a protein which may help to unravel the DNA. Genetic analysis has previously shown that the product of gene 32 of phage T4 is required for the replication and recombination of the phage DNA. This gene is unusual among those which affect T4 DNA metabolism because its product is needed in stoichiometric amounts and therefore does not seem to act catalytically. When *E. coli* cells are infected with phage T4, many proteins are synthesized which can bind DNA; the gene 32 product has been identified among them by the changes which take place in it when the infecting phage is mutant in this gene.

The protein is a single polypeptide chain of about 35,000 molecular weight which binds strongly to single stranded DNA. Because the binding decreases as the salt concentration is increased, ionic forces probably play an important part in the interaction. Binding of the protein to DNA is cooperative—that is, its affinity for DNA increases as the amount of protein present increases. This suggests that neighbouring protein moieties on the DNA interact with each other; as the protein is about 120 Å long and one binds for every ten nucleotides (70 Å) of DNA, adjacent proteins seem to overlap physically.

Both denaturation and renaturation of DNA take place more readily *in vitro* when gene 32 protein is added. The function of the protein in replication *in vivo* may be to help unwind the DNA at the replicating fork and align it in some particular way. Indeed, Alberts and Frey suggest that the T4 replicating structure may contain several gene 32 proteins that hold the DNA in a unique tertiary structure. Presumably, the DNA polymerase itself and some of the other T4 proteins concerned with DNA metabolism would also be part of this replication complex. The protein may also have another function; it could create regions of local denaturation in DNA as one of the first steps in separating the two DNA strands at the sites where genetic recombination is to occur.

MEMBRANES

Taking the Bio out of Biochemistry

from our Molecular Biology Correspondent

It is a healthy inclination to try to simplify biological systems by reducing the number of chemical components. It is also true, by and large, that time has not dealt too kindly with the outcome of much of the work on such models as synthetic polypeptides, which at

one time seemed to many people to be the key to understanding protein structure. Deeper understanding, it seems, is all too often bought at the expense of biological cogency, but model systems have had their successes. With so many beady eyes now swivelling towards biological membranes, it is not surprising that many of the more quantitatively inclined are occupying themselves with altogether simpler objects than red cells or bacteria, with their myriad proteins and lipids. In particular, it has become apparent that the bimolecular leaflets formed by lipids or their analogues have many structural and dynamic properties in common with real membranes. Just how far it will be profitable to press the analogy is not yet clear, but some features of striking interest have without doubt emerged from work on such models.

A polished study, using spin-labels, on the structure of a bilayer phase, composed of sodium decanoate, *n*-decanol and water, has been published by Seelig (*J. Amer. Chem. Soc.*, **92**, 3881; 1970). The bilayers form liquid crystals, which can be oriented homogeneously between glass plates, and can therefore be introduced into the magnet gap of an electron spin resonance (ESR) spectrometer with known orientation. The anisotropy of motion of a spin-label (that is, a molecule containing an unpaired electron) in the bilayer can then be defined in detail, and the well developed theory of the method exploited to best effect. The form of the ESR signal in fact shows a gross dependence (in terms of the magnitude of the hyperfine splitting) on orientation of the sample in the field. From the results one can deduce that a spin-labelled steroid, with a rigid skeleton, introduced into the bilayers, settles precisely along the optical axis that defines the line of the paraffin chains. The separation of the components of the spectrum corresponds to that found in decane solution, which shows that the label is deep in the hydrocarbon filling of the sandwich and from the nature of its restricted motion it follows that the hydrocarbon chains are parallel and rather extended, but engaged in rapid rotation. With a label attached to a fatty acid chain at different points, there is similar anisotropy, which varies with the position of the label, for with the carboxylate group lodged at the surface, the flexibility of the chain will ensure that the freedom is greatest at the far end. The chain, however, remains essentially extended, as is believed to be the case in liquid hydrocarbons. The smooth change in the spectrum with the separation of label and ionic group indicates that the polar boundary is diffuse rather than abrupt. Seelig has gone a step closer to membranes by looking also at his labels in dispersed phospholipid. The bilayers are evidently closely similar to those of the primitive system, though the presence of double-bonds seems to confer greater rigidity.

A rather similar system, in which a steroid-borne label is introduced into oriented phospholipid bilayers, has been examined by Butler *et al.* (*Biochem. Biophys. Res. Commun.*, **40**, 770; 1970). The chief message of this work, however, concerns the effect of cations, which are found to increase the anisotropy to an extent depending on their charge. It is conjectured that the ions—calcium and magnesium, for example, in physiological concentration—act by diminishing the charge repulsion between the phospholipid heads, thus allowing the bilayers to contract, so as to constrain the motion of the hydrocarbon chains.

Another simple, non-physiological and therefore tractable, material is the macrocyclic antibiotic, non-actin, which is known to promote the passage of potassium through membranes. Prestegard and Chan (*J. Amer. Chem. Soc.*, **92**, 4440; 1970) have followed the binding of alkali metal ions to this molecule using nuclear magnetic resonance (NMR). They find that in a non-aqueous solvent sodium, potassium and caesium ions are bound with rather similar association constants, the ring being evidently flexible enough to accommodate each with little strain. When water is added, however, the binding constants for sodium and caesium fall by two orders of magnitude, that for potassium by a mere factor of four. From the NMR spectra, which show no appreciable differences in the nonactin geometry whether the ions are taken up from wet or anhydrous solution (whereas the adjustment of the ring when it binds the large caesium ion can be clearly distinguished from its accommodation to sodium or potassium), it is deduced that the ions must bind without their hydration shell. Thus it follows that it is the relative energies of hydration which determine the large differences in affinity of the cations for non-actin. This kind of factor may well have a general bearing on ion sequestration and transport in membranes.

NUCLEIC ACIDS

Fragments and Initiators

from a Correspondent

A PUZZLE which provoked many topological exercises in molecular biology concerned the way in which DNA could be replicated in cells without getting in a terrible tangle. Okazaki and his colleagues demonstrated in phage-infected and uninfected bacteria that after brief pulses of radioactive precursors, the label is found in short pieces of DNA. These are then presumably linked together, to form the long chromosomal strands. Similar findings showed that discontinuous DNA synthesis probably also occurred in animal cells.

Such a scheme predicts the existence of a polynucleotide ligase to join the pieces together, and such an enzyme has been isolated from both infected and uninfected *E. coli*, and gene 30 of phage T4, which is required for DNA synthesis, has been identified as the structural gene of the ligase. Okazaki and others showed that when cells infected with T4 carrying a temperature-sensitive mutant of gene 30 were exposed to non-permissive temperatures, the short pieces of DNA accumulated. But Kozinski found no such fragments in infected cells labelled for long periods, if synthesis of a phage-induced endonuclease was blocked by chloramphenicol administered shortly after infection. He argued that the short Okazaki fragments arose from endonuclease action rather than discontinuous replication.

Iwatsuki and Okazaki have released a counter-salvo (*J. Mol. Biol.*, **52**, 37; 1970). They confirmed that addition of chloramphenicol 30 minutes after infection inhibits the formation of endonuclease, and prevents hydrolysis into short pieces of DNA labelled for up to 5 minutes. But when pulses of 10 seconds are used, the presence or absence of chloramphenicol makes no difference to the size (about 9S) of the short fragments produced, and such material continues to accumulate

with 20 or 40 second pulses in the absence of functional ligase. Therefore it seems that the pieces of DNA synthesized during short periods are not attributable to the action of endonuclease. If molecular biologists feel that they invented the subject themselves, they would probably be happier with their handiwork if the Okazaki mechanism remains as the way in which DNA is replicated.

In the same issue of the *Journal of Molecular Biology* (52, 91, 107), Mushynski and Spencer report the occurrence of pyrimidine tracts in the DNAs of phages T7 and λ , which they have studied in an effort to come to grips with the initiation points (promoter regions) of DNA-directed RNA polymerase transcription.

Only one of the strands of T7 DNA (the r strand) is transcribed *in vivo*, and the same strand exclusively binds G-rich polymers. Therefore it has been suggested that the promoter sites are pyrimidine runs in the DNA. Mushynski and Spencer separated the strands of T7 DNA, and obtained the pyrimidine tracts from each strand by degradation of the DNA with formic acid and diphenylamine. The tracts were fractionated into isostichs by chromatography on DEAE-cellulose. They found that the longest pyrimidine isostichs, containing eleven, twelve and thirteen nucleotides, arose exclusively from the r strand. But shorter stretches of pyrimidines were present in both strands. They noted that the isostichs containing twelve and thirteen nucleotides have together twelve components, equal to the number of mRNA species known to be transcribed off the T7 genome *in vivo*. The isostich 13 has seven components, and it has also been found that there are seven tightly binding promoter sites on T7 DNA. Therefore Mushynski and Spencer speculate that these longer pyrimidine tracts may indeed be the promoter sites, and also the areas responsible for poly G binding. In all cases, runs of C would be broken up by T residues, however. Equally they cannot exclude that shorter C-rich tracts interspersed with purines could both bind poly G and be potential promoter sites, except that they occur commonly in both strands. One observation which correlates with their suggestion that the long pyrimidine oligonucleotides are likely to be the regions specifically binding poly G is that λ DNA, which contains several A-rich purine tracts (deduced from the complementary pyrimidine oligonucleotides), is able to bind poly U, whereas T7 DNA, lacking these tracts, does not.

To make further progress, it will be necessary to isolate and characterize the authentic poly G binding sites, and this should be possible with the techniques now available.

TRANSPLANTATION

Successes and Problems

from a Correspondent

AN opportunity to review the progress and value of transplantation was provided by the international congress of the Transplantation Society held in the Hague during the first week of September. There was general agreement that kidney grafting is now an established and useful procedure; more than 4,000 transplants have been performed. The longest surviving patient, who received the kidney from a twin, is well after fifteen years. Although there have been

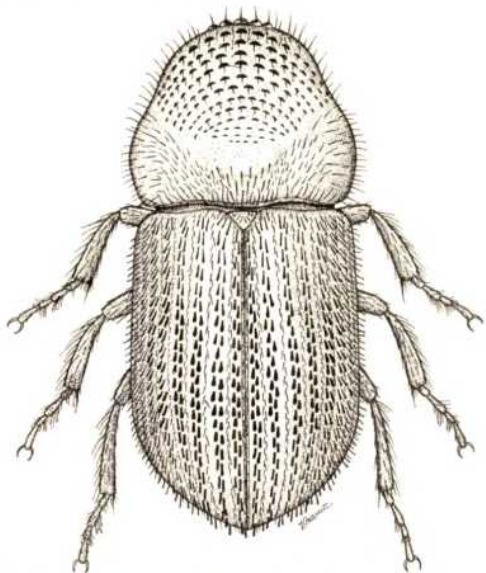
no important conceptual advances in kidney grafting during the past few years, results continue to improve as experience in the care of patients increases. The survival of grafts for one year is nearly 80 per cent from related donors and 50 per cent when donors are dead and unrelated to recipients. Twenty-two of the 165 hearts which have been transplanted are functioning. Twenty-six of the patients concerned survived for more than a year, and sixteen are alive after one to two years. The recipient who has survived longest, a patient of Dr R. R. Lower (Richmond, Virginia), is in very good health twenty-five months after the operation. A few patients with liver grafts are also surviving after more than a year. There has been little success so far with grafts of other organs, but after many earlier disappointments, successful engraftment of bone marrow has been achieved in patients with aplasia of their own blood forming tissue.

Serological tissue typing has been clarified technically, but although, as Dr P. Terasaki (University of California, Los Angeles) reported, a perfect match occurs in approximately 25 per cent of grafts between brothers and sisters with excellent results, such a match is rare between unrelated individuals. It is unfortunate that although degrees of mismatch can be determined, this ranking is not useful for predicting the outcome of organ grafts, except in perfect or nearly identical matches. Thus, because it is difficult to avoid rejection by matching there is a great need for better methods of overcoming rejection. Antilymphocyte serum (ALS), which is an extremely potent immunosuppressant experimentally, has proved difficult to obtain in an efficacious and non-toxic form. Nevertheless, hopes were expressed that improved purification and assay methods would produce useful ALS preparations, probably as adjuncts to the conventional immunosuppression of azathioprine and corticosteroid drugs.

Several experimental models described at the meeting have succeeded in part in satisfying the eventual goal of transplantation research, which is the specific abrogation of the immune response for the organ graft, without increasing the liability to serious infections. These models, in pigs, rodents and dogs, were described by Professor R. Y. Calne (University of Cambridge), Dr J. R. Salaman (London Hospital) and Dr R. E. Wilson (Peter Bent Brigham Hospital, Boston). At present the results obtained by using antigenic extracts from the donor to produce specific graft acceptance are unpredictable, and what is true for a graft of a certain tissue in a given species is often not applicable with a different organ in another species. Progress, however, has been encouraging and further studies along these lines may lead to predictable and safe methods of making an individual accept an organ graft without the necessity of prolonged treatment with potentially dangerous agents.

The idea of a bank of perfectly preserved organs stored for transplantation is still far from fulfilment. Simple cooling slows the deterioration of isolated organs and will allow twelve hours of kidney preservation, during which time tissue typing can be performed and the organ can be transported to an appropriate recipient. Complicated equipment that perfuses the organ with an artificial circulation can extend the period to twenty-four hours, according to Dr F. O. Belzer (University of California, San Francisco). But the heart, lung and liver are more sensitive and cannot be preserved for so long.

ENTOMOLOGY

Enemies of Trees

Two new species recently turned up among a consignment of bark and timber beetles presented for identification at the Plant Protection Research Institute in Pretoria. One of the hairy monsters was *Lanurgus oleaeformis*, shown here considerably larger than life (normal length is about 3 mm). K. E. Schedl, who has described the new species in the *Annals of the Transvaal Museum* (28, 177; 1970), suggests that South African foresters should beware of the damage that these beetles and their relatives could do.

RIBOSOMES

Slower Progress Now

from a Correspondent

IN the wake of the recent international biochemistry congress (*Nature*, 227, 1195; 1970) a ribosome workshop was held in Jöhny-sur-Vecy, Switzerland, from September 10–11, under the auspices of the European Molecular Biology Organization. The general atmosphere was of a period of consolidation after the recent advances in the understanding of ribosomal assembly and the structure of ribosomal RNA. Nevertheless, in spite of the absence of any spectacular advances, some elegant pieces of work were described.

In the session on ribosome assembly, Dr A. Bollen (Brussels) described how he demonstrated that some of the double-helical regions in the 16S ribosomal RNA are involved in the attachment of ribosomal proteins within the 30S particle. He reconstituted 30S particles, using 16S RNA complexed with ethidium bromide, which specifically interacts with the double-helical sections. The attachment of the "core" proteins displaces the dye (which is followed by the decrease in fluorescence). Dr C. Kurland (Wisconsin) presented important quantitative results showing that the attachment of certain "core" proteins to the 16S RNA is an entirely specific process. Dr P. F. Spahr (Geneva) examined the ability of various proteins from the 50S particle (from *E. coli*) to bind to two large fragments of the 23S ribosomal RNA, with sedimentation coefficients of about 13S and 18S (produced by mild enzymatic hydrolysis carried out on the 50S particle; fingerprinting showed that the larger fragment probably arises from the 3'-terminus). A few

of the proteins bound specifically to one or the other of the fragments, but most displayed some binding to either section, suggesting that the proteins might recognize multiple binding sites, widely separated from each other in the primary structure.

When ribosomal RNA was the topic for discussion, Dr Jordan (Marseilles) described his studies of the susceptibility of the 5S RNA to partial enzymatic hydrolysis. He was able to split the molecule in two (with a cut at position 41–42) and then recombine the halves. He also found that this point was particularly prone to hydrolysis when subjected to mild digestion of the RNA within the 50S particle, suggesting it is especially accessible to the nuclease. He noted that the point of hydrolysis is adjacent to the sequence —CCGAAU—, which could base-pair with the tRNA sequence GT ψ CG. The audience swallowed the implication with a large pinch of salt. Dr J. P. Ebel described some recent work by the Strasbourg group on the 16S RNA from *Proteus vulgaris*, which is similar in primary structure to that of *E. coli*, but has some differences which seem to be more frequent in some parts of the molecule than in others. Dr H. Wittmann (Berlin) produced a complete table showing which of the 30S ribosomal proteins correspond to each other in the classifications adopted by the various groups working on them. This mightily reassured the faithful. Other highlights of the session on ribosomal proteins included the work of Dr R. Brimacombe (Mill Hill), who digested 30S particles with ribonuclease and fractionated the resulting ribonucleoprotein fragments by electrophoresis on polyacrylamide gel. He extracted the proteins from the different fractions, and was able to show that some of the fractions were particularly enriched for certain of the proteins. Dr W. Moller (Leiden) described some of the properties of an acidic protein from the 50S particle, which is probably the only ribosomal protein to occur with a frequency of two copies per ribosome. He wondered whether it was in any way involved in directly interacting with tRNA, in the light of the existence of two tRNA attachment sites on the 50S particle.

A session on ribosomal structure featured largely the gross morphology of ribosomes, their degree of hydration, and so on. One of the most promising long term approaches must be X-ray diffraction studies. Dr W. Hill (Montana) described what he has found using low-angle X-ray diffraction and suspensions of the ribosomal particles. He estimated that the hydrated ribosomal particles from *E. coli* had the following dimensions: 30S: 55 \times 220 \times 220 Å; 50S: 115 \times 230 \times 230 Å; 70S: 135 \times 200 \times 400 Å. To explain how the two sub-particles could give rise to a particle as compact as the 70S ribosome, he suggested that the 30S particle is wrapped over one "side" of the 50S particle, as a sort of cap.

PHOTOELECTRON SPECTROSCOPY

Enthusiasm for New Technique

from a Correspondent

PHOTOELECTRON spectroscopy, which has created a considerable stir in several disciplines, provided the theme of a meeting held in Oxford on September 14–16. The emphasis of the meeting, organized by the Institute of Physics and the Physical Society in collaboration with the Theoretical Chemistry Group of the Chemical

Society, was on the physical side of the subject. The technique consists of measuring the kinetic energy spectrum of electrons ejected from molecules by high energy monochromatic photons. If rare gas resonance radiation is used as the photon source, then outer valence electrons are ejected giving a spectrum which permits a study of the structure and bonding of molecules. This direct study of the orbital structure of molecules was described by Dr D. W. Turner (University of Oxford) who founded the subject.

Core electrons close to the hearts of the atoms on a molecule can be ejected by X-radiation. The surprising and exciting result, that the ionization potentials of inner electrons experience a chemical shift dependent on the environment of the atom concerned, was discovered by Dr K. Siegbahn in Uppsala. At the meeting this topic was introduced by Dr B. Lindberg (Uppsala) who described a diversity of examples illustrating how the chemical shift is directly related to the charge density at the atom from whence the electron is removed. Even using the very simple ideas of charge distribution given by Pauling, the spectra were shown to help in solving various chemical problems.

To judge by the applications cited, the technique has developed a long way already. As well as many examples of the study of small gaseous molecules, there were applications of ultraviolet photoelectron spectroscopy to inorganic complex molecules, from Drs D. R. Lloyd (University of Birmingham) and A. F. Orchard (University of Oxford), to transient species from Dr N. Jonathan (University of Southampton) and even to herbicides and pesticide molecules from Dr A. D. Baker (Swansea). The range of application of X-ray photoelectron spectroscopy was even wider, including solid state effects as well as examples in chemistry and biology.

The commercial world has quickly grasped the possibilities of this relatively new technique, and some of the instruments on show during the conference were so new that they did not have prices. As so frequently happens when an experimental innovation startles the scientific world, the theoreticians have followed up with explanations of the observations. Apart from giving a new lease of life to the molecular orbital, the technique gives the opportunity for considering several interesting theoretical effects. The Jahn-Teller effect can be very important, as Professor R. N. Dixon (University of Bristol) showed. He and Dr A. W. Potts (London) indicated that there are several possibly significant effects which cannot be disregarded by those who study larger and larger molecules.

The overall impression of the conference was largely reminiscent of nuclear magnetic resonance ten years ago, for here is a technique which clearly has almost limitless applications in chemistry and related subjects. At present everyone is leaping on the bandwagon, studying everything in sight (or perhaps in bottles), but the prizes could be tremendous, and so it is all worthwhile.

GLASSES

Structures and Structons

from our Materials Science Correspondent

MICROSCOPIC and macroscopic approaches were neatly balanced in the varied programme of the international

conference on the physics of non-crystalline solids held at Sheffield from September 14 to 18. Even metallic glasses, a very recent topic, received the accolade of inclusion in the form of a single contribution, concerned with visco-elastic properties. Two Russian contributions were listed and duly not presented.

A good deal of attention was devoted to phase separation in oxide glasses, and one enthusiast, Professor Rustum Roy (Pennsylvania State University), even suggested that the tendency to phase separation is a general property of all glasses. Dr G. H. Frischat (Max Planck Institute, Würzburg), who has studied tracer-diffusion in $\text{Na}_2\text{O-SiO}_2$ glasses, was able to show that when phase separation took place the diffusion profile could be analysed into two constituent standard profiles, while Mme M. Prod'homme (Paris) demonstrated the time variation of viscosity in borosilicate glasses undergoing phase separation. Professor D. E. Day (University of Missouri-Rolla), however, in a comprehensive study of mechanical relaxation in mixed alkali silicate glasses, established a relaxation peak, due to the coupled and mutual re-orientation of dissimilar alkali ions, which was not affected by phase separation in a $\text{Li}_2\text{O-Na}_2\text{O-SiO}_2$ glass.

Professor B. E. Warren (Massachusetts Institute of Technology), reviewing his recent X-ray work on the structure of pure SiO_2 and B_2O_3 glasses, emphasized that developments in technique had so improved resolution that the study of glass structure by this approach now needed to start again from the beginning. He showed that, for the first time, distributions of bond lengths and bond angles could be established. Dr A. J. Leadbetter (University of Bristol) showed how neutron-scattering can improve on some aspects of the X-ray approach, especially because of the large values of $\sin \theta/\lambda$ accessible and the easier interpretation of the raw data. He presented results of X-ray and neutron studies of BeF_2 and As_2Se_3 glasses.

The experimentally biased contributions were complemented by the veteran chemist, Dr M. L. Huggins (California), who surveyed his "structon" theory, as applied specifically to boric oxide glasses. Structons are groupings of B and O ions of different multiplicity, oxygen atoms being singly, doubly or triply bonded, so that structons of different sizes have different net charges, which have to be compensated by neighbouring structons. The distribution of structon types is a function of a composition and can be directly related to the results of X-ray and nuclear magnetic resonance examinations. It remains to apply this valuable quantitative approach (a kind of Pauling theory for glass-bonding) to the interpretation of physical properties, such as viscosity, which are a function of the concentration of non-bridging oxygen bonds.

An intriguing new experimental approach to glass structure was described by Drs J. Götz, C. R. Masson and K. M. Castelliz (National Research Council of Canada), who showed how ionic groupings such as SiO_4^{4-} , $\text{Si}_2\text{O}_7^{6-}$ and so on can be extracted from a silica glass by trimethylsilation followed by gas-liquid chromatography and mass spectrometric assay of the relative incidence of the groupings in the original glass.

Of several industrial contributions, perhaps the most immediately useful was from Drs P. C. Schultz and H. T. Smyth (Corning Glass Works), who prepared a series of $\text{SiO}_2\text{-TiO}_2$ glasses by flame-photolysis of

chlorides and so obtained glasses with virtually zero expansion coefficients between 0° and 400° C.

COMPUTATIONAL PHYSICS

Collisions and Tides

from a Correspondent

MANY of the techniques and difficulties of computational physics are shared by people working on topics as diverse as plasma physics, atomic structure, lattice statistics, hydrodynamics and semiconduction transport theory. This was clearly illustrated by the conference on computational physics held by the Computational Physics Group of the Institute of Physics and the Physical Society which took place in London from September 7 to 9.

One theme was the theoretical determination of important physical parameters, and vital contributions came from Professors P. G. Burke (Queen's University, Belfast) and M. J. Seaton (University College, London). Burke's group at Belfast has developed a general set of codes to describe atomic collisions and scattering cross-sections. For a given atom, five procedures are followed on the computer: the determination of relevant atomic wave functions; the calculation of oscillator strengths and hence the determination of the polarizability of the atom; the effect of long range atom-atom interaction, and the calculation of electron-atom scattering cross-sections. Machine independent self-contained modules have been developed, which it is hoped other workers may usefully employ. Seaton illustrated the importance of this work in the interpretation of solar and astrophysical data.

Mr C. L. Pekeris (Weizmann Institute, Israel) described the results of a two dimensional programme to describe the tides in the oceans of the world. A massive calculation using 180,000 mesh points had been used to obtain quantitative agreement with the known motion of the tides. Hence the frictional drag of the tides on the Earth's rotation was empirically determined and correspondingly the acceleration of the Moon's revolution about the Earth could be obtained. Several previously undetermined features of the Earth's tides were found, in particular the existence of a null point in the South Atlantic.

Several contributions were concerned with plasma physics and magnetohydrodynamics. Dr D. E. Potter (Imperial College, London) illustrated results of two dimensional time dependent magnetohydrodynamic phenomena, results in quantitative agreement with experimental plasmas. He said that in the next few years it could be possible to describe the structure of the magnetosphere, of the Earth's dynamo problem and of toroidally contained fusion plasmas. In a series of contributions, Drs R. Peckover, K. V. Roberts (UKAEA, Culham), M. Petravic, and G. Kuo-Petravic (Oxford) illustrated how the programming and structure of such very large complex computer codes could be greatly simplified by symbolic methods. Symbolic languages have been developed so that the simple and clear notation of vector calculus can be used almost directly in programming the computer, and with very little loss of computer execution time.

There was a consensus of opinion that while many of the equations of classical and quantum physics are well known, the limitations of analytic mathematics

prohibit an understanding of many significant phenomena. The development of models on the computer, and the observation of their evolution in time, will clearly make a significant contribution to the development of all branches of physics in the future.

GAS DISCHARGES

Breakdowns and Thrusters

from a Correspondent

GAS discharges were the topic for discussion at an international conference held in London from September 15 to 18, under the auspices of the Institution of Electrical Engineers, the Institute of Physics and the Physical Society, and the Institute of Electrical and Electronics Engineers.

A session on pre-breakdown phenomena again revealed that the magnitude of breakdown voltages in uniform or near-uniform field configurations cannot usefully indicate either the relative importance of different secondary processes in Townsend growth or the nature of the transition to a streamer process. Discussing the divergent field gaps which are important in engineering, Dr T. E. Allibone (formerly of Central Electricity Generating Board) pointed out that experimenters remain indispensable in the absence of any quantitative model of flashover. Professor V. Popkov (Khrzhanovsky Power Institute, USSR) emphasized that the flattening of the breakdown voltage versus gap clearance characteristic made prospects of power transmission at levels above 1,200 kV extremely remote. Even at two-thirds of this level, the problems associated with corona noise and power loss are formidable.

The even more contentious subject of electrode phenomena aroused lively discussion of cathode-spot emission mechanisms and retrograde arc motion. A useful contribution to the physics of cold cathodes was made by Dr A. E. Guile (University of Leeds), Professor T. J. Lewis and Mr P. E. Secker (University College of Bangor) when they discussed the role of surface oxide layers at the cathode. The very large current density observed at cathode spots (up to 10^{12} A m⁻²) was accounted for in terms of a tunnel (autoelectronic) emission of electrons through the oxide coating, this emission being caused by an electric field of about 10^9 V m⁻¹ created by positive ion deposition on the oxide. They also claimed that electron velocity components parallel to the cathode surface could account for retrograde motion in a transverse magnetic field. It would seem difficult, however, to omit column effects entirely from any account of retrograde motion.

Another cathode spot mechanism, first proposed by von Engel and Robson, has been investigated by Mr A. J. T. Holmes and Dr J. R. Cozens (Imperial College, London). The release of electrons by the action of metastable atoms at the cathode was found to be observed best by measurement of the glow-to-arc transition voltage. A useful by-product of this work is that gas density increased at the cathode which served to increase the transition voltage—a possible new approach to arc inhibition.

The SERT II mercury ion thruster for space propulsion was described by Mr H. J. King (Hughes Aircraft). A Penning discharge produced a 20 A electron current, from which a 4 A ion beam was extracted. Subsequent acceleration and neutralization of the beam provided 0.028 N thrust, at an exhaust velocity of 30 km s⁻¹ and

81 per cent fuel efficiency. The application of ion propulsion to the raising of communication satellites to synchronous orbit would warrant their development in the United Kingdom. The confidence of Dr R. S. Pease (UKAEA, Culham) in the eventual success of controlled nuclear fusion, partly because of the 10 ms confinement times achieved in Anglo-Soviet experiments, contrasted with the catalogue of difficulties which Dr D. Balfour (IRD Co., Ltd) was obliged to report for MHD generation. The materials problems associated with this technology are being attacked, however, and one awaits with interest news of the performance of the 25 MW open-cycle system which is being constructed in Russia.

LABORATORIES

Atomic Architecture

from a Correspondent

UNIVERSITY architects were in the audience at a symposium on the design of laboratories for work with radioactive materials held by the British Radiological Protection Association at Birmingham on September 18. Effective communication between architect, builder and user is crucial when the laboratory must handle large quantities of unsealed (or open) radioactive substances.

Mr J. M. Rees (Department of Employment and Productivity) discussed the theoretical grading of laboratories originating in the International Atomic Energy Agency Safety Series Booklet 1. Three grades of laboratory were defined in increasing levels of specialization which required minimum standards of finish, fitting and facilities. Although this helps to ensure that work is not done in a seriously inadequate laboratory, Rees said that it was equally important to avoid costly and elaborate facilities for elementary work. There was general agreement that high grade laboratories were difficult to incorporate in a multistory block. A ground floor suite of laboratories gave least difficulty for drainage of radioactive waste and permitted high load bearing floors to carry shielding and other heavy equipment.

Ventilation of laboratories and discharge of fumes have been studied at Leeds by wind tunnel tests of a building model. Mr K. Everett (University of Leeds) said that the exhaust fumes should be discharged at a height 50 per cent greater than that of the building. This ensured penetration of the turbulence envelope around the building and discharge of the fumes into a dispersal zone away from the building.

Dr D. Hughes (University of Leeds) stressed that laboratory design must allow for accidents. Because spills of radioactive liquids are inevitable, surface finishes must suffer the rigours of frequent decontamination. Mr J. W. Lucas, describing a bewildering array of surface finishes, said that good planning could prevent excessive demands on these surface finishes. He offered a simple procedure for selection of the finish which did not suffer from the complex decontaminations and counting procedure of the British Standards Institution test (BS4247, Parts 1 and 2, 1969).

The choice of stainless steel (preferably EN58J grade) for lining fume cupboards was unanimous. The Department of Employment and Productivity has produced an advisory note on fume cupboards,

or partial enclosures, which should be read by all designers and users of this equipment. Mr P. J. Hewitt (University of Bradford) favoured the by-pass type of fume cupboard which maintains a satisfactory velocity of air through the open sash, substantially independent of each opening and operator movement. He reminded users that certain fume cupboards with a by-pass to the laboratory must be operated continuously if they are to be regarded as total enclosures for the more toxic radioactive substances.

ION IMPLANTATION

Technique goes Critical

from a Correspondent

THE current interest in the controlled introduction of impurities into solids by implanting them from an accelerated ion beam was reflected in the large and enthusiastic attendance at a conference held, by the Institute of Physics and the Physical Society with the Institution of Electrical Engineers, in Reading from September 7 to 9. As a practical technique for semiconductor doping the topic was summed up neatly by Dr J. Beale (Mullard Research Laboratory, Redhill, Surrey), who feels that ion implantation has now gone critical and there is no barrier, technical or economic, to its use. He emphasized that as well as its application in semiconducting devices the technique provides a means for making good resistors in integrated microcircuits. The designers of such circuits have thus been saved from abandoning their practice of using resistors as the stable elements in circuits.

Many contributors dealt with the underlying science of ion implantation. Considering the concentration gradients that exist in implanted specimens, Dr G. Dearnaley (Atomic Energy Research Establishment, Harwell) emphasized the relevant features of ion range distributions in solids, particularly the dependence of the electronic stopping power on atomic number. In carefully selected cases, such as Na^+ channelled in a Cu crystal, he has shown that surprisingly long ranges are possible, even up to 10 μm at a few hundred keV.

Once implanted, the ion becomes a foreign atom in a damaged solid, but several speakers emphasized that this is not necessarily a stable state thermodynamically, and the impurity atom is quite likely to be associated with other defects, or with other impurity atoms, in defect clusters. Many contributions made it evident that annealing after implantation caused defect rearrangements which further affected the physical properties. It is well known that ion bombardment to doses larger than 10^{15} ion/cm² can turn Si and Ge apparently "amorphous". But what is really meant by amorphous? This tricky point was explored carefully by Dr P. A. Whalley (Chelsea College of Science and Technology) drawing on his observations of evaporated or sputtered semiconductor films.

One of the high spots of the conference was a description by Dr J. A. Davies (Aarhus University, Aarhus, Denmark, and Chalk River Nuclear Laboratories, Chalk River, Ontario) of the techniques he and his colleagues have developed for using ion beams themselves as a probe to determine the state of an implanted crystal, to detect displacement damage and distinguish it from implanted atoms. He also showed how the location of the implanted atom relative to the crystal lattice can be deduced.

Scientific Responsibility

by
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This personal view of the responsibility of scientists for the consequences of their research is an abridged version of an address given as part of a programme, Responsibility of Scientists in Society, at the annual meeting of the Biophysical Society in Baltimore on February 26 this year.

We are all troubled by a paradox: the apparent triumph of the rational mind, of the scientific discourse as exemplified by us, its practitioners, and at the same time a rapidly expanding sphere of irrationality abounding in the world around us. For the first time in decades, scientists are being seriously questioned as to the primacy of their endeavours, the importance of their beliefs and their goals. Indeed, some go so far as to question the need for scientists to exist at all. These are serious questions, even though youthfully, and sometimes illogically, voiced in terms of misunderstanding or even hatred. But while today we scientists are being literally shouted at, the substance of the remarks aimed at us is nothing new—only the tone and volume have changed. Scientists themselves have often asked whether the scientific endeavour is doing right in its obligation to the world. We all know the woeful tales of many of the nuclear physicists, of the doubts that kept appearing during Einstein's last years. What I am trying to say is that what is being loudly proclaimed now by terrifying youth has already been stated, in a calmer, indeed more tragic form, by many scientists themselves: "what has science wrought?"

First, how do we think today about scientific research? We find principles that are exactly the same as those of a hundred or several hundred years ago: an objectivity in research, a disinterest in consequences, a disclaiming of values external to research itself. But these principles were formulated in an environment which was hostile to science, in which good and evil existed—a subjective universe where fact and fancy were often not clearly delineated, because fact was not grounded on observation or experimentation and fancy was the whim of the powerful and the rulers. I therefore propose that the highly touted principles arose, not only from tenets of philosophy or empirical methodology, but also from a practical need to survive. To exist in a hostile world of values, one had to have recourse to a world of no-values, to research with results that would not affect values or beliefs external to itself.

Now, of course, the scientists are the ostensible masters: research and technology set the standards and the values, and the outside world follows. Can scientists, in this situation, still claim a non-responsibility? Perhaps this would hold for technologists, but not for those engaged in pure scientific research, for by definition the latter has no merit except to its practitioners. I challenge this

idea that research can be separated from development, from technology; I believe all scientists know that what they do in the laboratory can and will have effects, sometimes immediate, sometimes delayed, on the world outside. The most famous example is, of course, the highly esoteric world of the high-energy physicist of several decades ago. Thus I see no greater immediate task, though it is a small step to take, than for scientists and their organizations to acknowledge their responsibility. But how?

Possible Solutions

I suggest here some possible answers, some of which may be considered outrageous, though I should like to point out the extremes simply to clarify the problem. I should stress that I do not consider myself to have the answers to this question of responsibility, but wish to provoke the reflexion that can lead to answers.

Consider the idea of an "oath". Two oaths have been circulating in the Berkeley-Stanford and the Columbia-New York City areas, and there may well be others. Both are pledges not to engage in war work. In some cases the situation is clear-cut; physicists should not work on nuclear bombs, and chemists and biologists should not develop agents of chemical and biological warfare. Indeed, in these cases I would go further, in that I would not acknowledge as fellow scientists those who consistently use their skills in the service of killing men. They should not be asked to meetings, and should not be allowed to publish their results. They could be free to do their work, but I think we have a right and a duty not to acknowledge them as fellow members of the scientific community.

But what is a scientist? Whom do we elect to membership in our societies? What should be their qualifications? To that definition of a professional as one qualified to do research and to teach by accepted standards, I think we should add a standard of responsibility. Such a standard would not allow us, for example, to call physicians those who would experiment on humans; to call microbiologists those who would develop lethal viruses; to call chemists those who would develop chemical weapons; to call physicists those who would build bigger and better bombs. To me these are clear-cut cases where we can draw the line between those we would call scientists and those we would not.

It is the ill-defined cases which make for the present

soul searching. Are we to condemn physicists who advocated testing of nuclear weapons without knowing of the genetic effects of radiation, or chemists who devised pesticides and herbicides without knowing of their ecological consequences? These scientists are not lost souls, evil, cruel men, or mere fools made knaves from ignorance. I submit that they, and we, know much less than we think, that our vaunted knowledge of physics and chemistry and biology is but the skin of the apple. We cannot really be faulted, because the structure of our science, its reductionism, has prevented us from seeing far consequences when we were only looking for immediate results. An oath taken in these circumstances is meaningless; it is no good asking scientists not to work on factors affecting nerve transmission because in an unforeseeable future these findings would be used as a basis for a nerve gas. All working scientists know it is hard enough to make predictions for just a few years ahead in one's own immediate field of research, without trying to bother what others, in this case adventurers and politicians, might make out of it. When the consequences of research are clearly immediate, the oath taking, the ostracizing of scientists, the raising of standards of responsibility within societies are meaningful gestures; but when the consequences are unforeseeable, such acts obscure the real factors involved in the social impact of science.

Foreseeable Consequences

However unforeseeable some research is, a good deal is on the borderline in that we can see, not always clearly, what its consequences might be. Indeed, we can even go so far as to plot alternatives: thus, if research continues to increase the efficiency of yield from nuclear power, we can weigh the increased radiation from nuclear power plants against the chemical pollution from conventional energy-transforming systems. One solution to this sort of question is that what happens to the world as a result of research and technology is the responsibility not only of the scientists but of everybody, and that the special responsibility of a scientist is to insure that the public are informed about alternative possibilities. In this way, about ten years ago, there arose the scientific information groups, initially as a result of the public clamour about radiation hazards from nuclear weapon testing, but now embracing all sorts of environmental hazards.

Suppose, then, that each scientist takes ten per cent of his time away from research or teaching, to inform the public about scientific matters. One does not need to be an expert; one needs only know more than the general public. Scientists know how to reach the pertinent literature, and how to appraise it, and can easily learn how to condense it and present it knowledgeably to the general lay public. Each scientist can become an explainer to the public, concentrating on problems where technology has confounded public expectations. In a democracy of equal voters, this task of scientists becomes a duty; it is a small enough measure to take when we think of our greater responsibilities for the world in which we live.

Redirection of Research

As an extreme, I would suggest further that technology has badly outraced the political and social means of handling the problems it generates, that research and development are so intertwined that the former leads invariably to the latter, and that, to give political methods a chance to work, or to devise new political methods, technological advance, and thus research, should stop.

A greatly modified version of this proposal is that the present research programme be abolished in favour of a redirection to cure the ills of technological gains. I think something like this is coming, whether or not scientists want it or are even asked about it. The US Secretary for Health, Education and Welfare, R. Finch, has clearly come out for a reassessment of medical funds, for a usage of funds which would give less for medical research and more for medical care. Director McElroy of the US National Science Foundation (NSF) has clearly formulated the increased role of the foundation in looking towards a solution of technological problems by directing its research funds towards the troubled areas. The US National Bureau of Standards is experiencing a change of emphasis, delving deeper into problems related to consumers rather than to industry. Most important is what is happening in the Federal Bureau of the Budget, in that goal-directed government bureaus in charge of research funds are being asked to apply direct connexions between the contemplated research and the goals of the agency. All told, basic research will almost disappear from the Washington science scene. Biologists, for example, will no longer obtain money by begging the question of the "cancer cure". This in itself is not such a bad thing if it brings back an honest approach to the fund granting agencies. What I fear is that, worthwhile as these goals might sound, the scientists will be altogether left out of formulating them. Probably they will no longer be the sole determinants of what the research aim is to be, but they should at least be consulted.

My answer to the immediate problem is to stress the educative value of research, the idea that science and research are a part of historical and cultural heritage, with a structure that should be handed on to future generations, that scientific research is not really about travelling on the Moon, or faster over the oceans, or about more energy, or better food, or population control, but that it is a part of the civilizing experience, a book of learning. It is this which the citizens of the world should support, for it is part of what makes us man and not beast.

Long Term Answer

My answer to the long term problem is to make sure that scientists are not entirely left out of the decision making about research and its applications. The special responsibility of the scientist is not only to point out the scientific merits of a piece of research but to try also to say something about its social risks and social merits. Risks there must be, for we are human beings and not gods, but the outcomes of immediate risks can be minimized and those of unavoidable risks can be slowed down. In this connexion panels of the American National Academies of Engineering and of Sciences have been issuing reports on technology assessments (the processes by which the probability of new technologies can be foretold) and the possible formulations of alternative courses of action based on these probabilities. I do not think very highly of these studies, for they tend to end up as studies of risk assessments; by the very nature of large scale technologies, the conclusions of many of these studies are cast in the form of evaluating minimum or what may seem to be insignificantly small risks, evaluations which may prove to be correct but can also be very wrong, and whose incorrectness will not be noticed until a grave situation has arisen. I need mention only pesticides, herbicides, gaseous pollutants or drugs. We simply

do not know enough, and in the foreseeable future will still not know enough, about the real world rather than the world of the laboratory.

I think we are looking at these problems the wrong way round. We are viewing technological advance in many fields as inevitable, and are deciding in effect which alternative technology will produce the least dangerous results. For it is always assumed that the chief goals of technological advance will be beneficial. Thus we will choose a technology which will put filters on car exhausts but we do not ask whether cars are always necessary; we decide to build more power plants and argue whether the fossil fuel burners or the nuclear transformers will be better for the environment, but we do not ask whether the increased energy output is really necessary, whether it is necessary to have a washing machine in every home and air conditioners in every room. In other words, technological advance has been haphazard, based on nothing but chance and market economies, and is thus quite out of keeping with the methodology of the scientific experiments which have led to it. This occurs in any technologically abundant society, whether the society be entrepreneurial as in the United States or planned as in the USSR. Technology, and responses to the problems posed by technology, have been based on perceived, superficial needs and not on real human needs.

More Research and Technology

Nevertheless, the idea of directed research has often been broached, though with the objective not of building a more natural habitat for humans but of undoing the damage caused by previous technology. Perhaps the most far-reaching of these proposals are those of Platt¹ and Weinberg². What they all say in effect is that the ills of

technology can be cured by more research and technology, the technology being directed towards commonly approved goals. The most ludicrous of these proposals is that of Roberts³, who suggests that space technology should be redirected towards terrestrial goals, when his goals turn out to be only more efficient ways of ravishing the Earth.

I would thus suggest that the present environmental mess is caused by rapid technological growth, driven on by haphazard research, during the past thirty years; that this research and development are inexorably linked; and that, in spite of this research, we scientists have only little understanding of the natural world around us. Given these beliefs, it seems to me to be disastrous to think that further refined technology, directed though it may be, will cure past technological mistakes. So is it to be the ultimate responsibility of scientists to society that they discontinue their existence as research scientists?

I conclude by pointing out the remarkable hubris of we scientists. We take pride that we alone are responsible; pride that we can undertake to make matters right; pride that we think we know enough to make for a perfect world; my pride that I can say this. Pride is one of the biblical sins; I think our greatest sin is to presume to know much more than we do, and even if we don't, we give the impression that we do, and so the world takes our tentative findings and makes them actualities, and this is where we stand today. Thus, we should be humble above all else, and realize that though we humans may be just a little below the angels, we are far from being gods.

¹ Platt, J., *Science*, **166**, 1115 (1969).

² Weinberg, A. M., *Science*, **167**, 141 (1970).

³ Roberts, W. O., *Science*, **167**, 11 (1970).

Extragalactic Variable Radio Sources

by

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Clues to the origin of the enormously energetic activity in quasars and galactic nuclei may come from observations of the varying radio emission from several objects outside the Galaxy. The varying signals from these objects often seem to come from areas which are only a few light months in diameter.

By the middle of the last decade, there was evidence from several different types of observation that some of the extragalactic radio sources have components far smaller than the typical extended radio clouds revealed by low frequency observations. Scintillation of radio sources observed through inhomogeneities in the solar wind indicated that there were sources with components smaller than a few tenths of one second of arc angular diameter^{1,2}. Extension of radio flux determinations to higher fre-

quencies revealed an upturn in the spectra of many sources at frequencies beyond 3 GHz. The natural interpretation of the increasing flux at high frequencies was that it was synchrotron radiation from a small source which had such a high surface brightness that it was optically thick to its own low frequency radiation^{3,4}. The inferred sizes were typically a few thousandths of a second of arc⁵. Further evidence for the existence of very small components was provided by the discovery of temporal variations, with time-scales of about a year, in the high frequency

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flux of several of the radio sources with upturned spectra⁶.

There are now some thirty known radio variables⁷. Most are identified optically with QSS, and some others with Seyfert and compact galaxies. Most of the radio variables are also optical variables. In fact, the optical activity of two radio sources, VRO 42.22.01 identified optically as BL Lacertae, and 3C120, a compact N-type galaxy, was noted long before the observations of their remarkable radio emission. There does not seem to be any correlation between the optical and radio variations, and not all optical variables are radio variables.

The strongest of the radio variables (the Seyfert galaxy 3C84, 3C120, and the quasars 3C273, 3C279, 3C345 and 3C454.3) have now been monitored regularly by many observers over frequencies extending to 88 GHz. These sources have been very active, producing about one radio burst per year. The data for 3C120 (Fig. 1) show the typical evolution of a burst⁸. At each frequency of observation there is a gradual rise and fall in the flux over time scales of several months. The burst is first detected at high frequencies. With increasing time the burst propagates to lower frequencies with successively decreasing amplitude. Such evolutionary behaviour of the spectrum of the burst can be understood in terms of a model developed by Shklovsky⁹, van der Laan¹⁰, and Kellermann and Pauliny-Toth⁷, in which the flux is

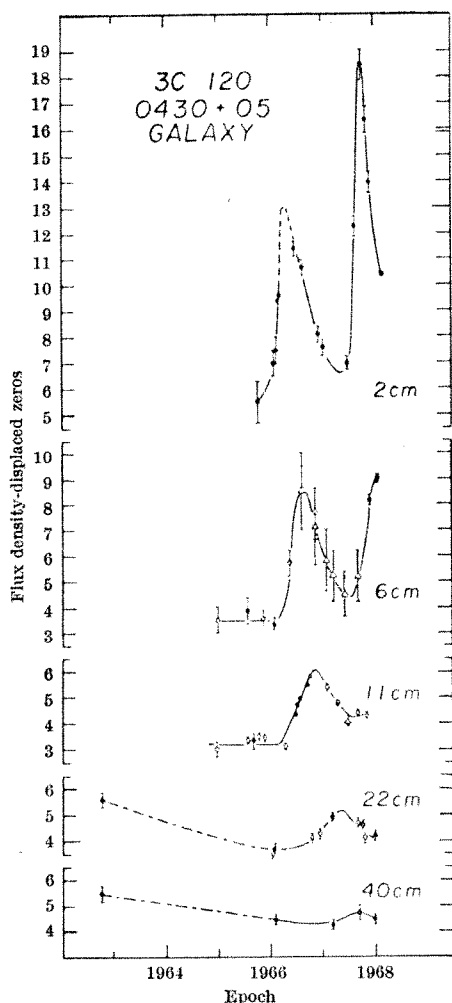


Fig. 1. The radio variations of the compact, N-type galaxy 3C120 (reproduced from ref. 8).

attributed to synchrotron radiation from a small, radially expanding cloud of relativistic particles and magnetic fields. At any age of the burst, the synchrotron radiation would be self-absorbed at frequencies below ν_{crit} , and in that portion of the spectrum the flux would be proportional to $\nu^{2.5}$. Above ν_{crit} , the source would be optically thin to its synchrotron radiation and the flux would be proportional to $\nu^{(1-\gamma)/2}$ where γ is the index of the inverse power law energy distribution of the relativistic electrons. Typically γ is around 1.5 in these sources so that the flux peaks at $\nu \approx \nu_{\text{crit}}$. If it is further assumed that the energy of the electrons varies adiabatically as the source expands, and that the magnetic flux is conserved during the expansion, the detailed spectral evolution of the burst can be predicted for comparison with observations. As the

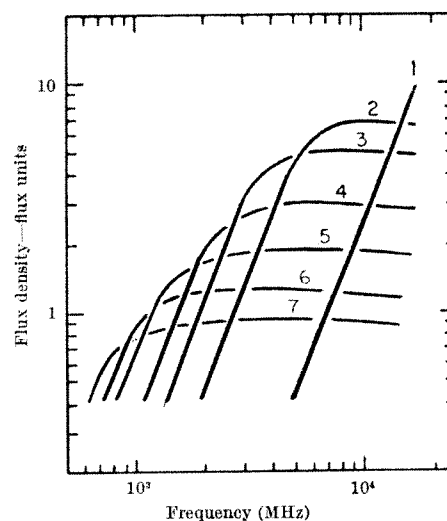


Fig. 2. The spectral evolution of the component of 3C120 variable during 1966-68 based on the data of Fig. 1. The spectra are presented at quarter year intervals starting at 1966.25 (reproduced from ref. 8).

source increases its surface area, the flux is expected to increase at frequencies below ν_{crit} , decrease at $\nu > \nu_{\text{crit}}$, while ν_{crit} itself decreases during the expansion (Fig. 2). The maximum flux observed at a given frequency is simply proportional to $\nu(7\gamma+3)/(4\gamma+6)$. If it is assumed that the source size increases uniformly with time, then the peak flux will occur at the frequency ν at a time $t \propto \nu - (\gamma+4)/(4\gamma+6)$. It has been demonstrated by Kellermann and Pauliny-Toth⁷ that observations of the evolving spectra of the bursts fit the model surprisingly well. In principle, observations of the time variations in the polarization of the synchrotron radiation should provide a further test of the model. Pacholczyk and Swihart¹¹ have shown that in a synchrotron self-absorbed source the degree of polarization is $3/(6\gamma+3)$, with the position angle of the polarization along the magnetic field, while it has been well known that in an optically thin source the degree of polarization is $(3\gamma+3)/(3\gamma+7)$, with the polarization perpendicular to the field. Observations of the polarization variations at 8 GHz in these sources have revealed very complex behaviour, with rapid fluctuations in both the degree and direction of polarization¹². Aller has pointed out¹³ that the largest of these variations is observed when the source is only barely self-absorbed so that they may be reasonably accounted for by different

parts of the source, with different magnetic field orientations, becoming optically thin. (There are, however, several sources^{14,15}, most notably VRO 42.22.01, which show extremely rapid fluctuations in both total flux and polarization that do not seem to fit the model at all.) These observations, and the apparent absence of free-free absorption, set strong limits on the amount of thermal gas that can exist in the region of the source.

The surface brightness at which synchrotron self-absorption sets in is proportional to $B^{-1/2}$, where B is the magnetic field strength, and the constant of proportionality is known theoretically. One can therefore calculate (except for a $B^{-1/4}$ dependence) the linear size of a source of known power. The expansion speed can then be inferred directly on the basis of the model, from the rate of variability. If, however, the expansion velocity v is relativistic, corresponding to a bulk Lorentz factor $\Gamma = (1 - v^2/c^2)^{-1/2}$ the rate of change of the angular size of the source is proportional to Γv because of the doppler aberration. Hence the time variations of the flux can be arbitrarily more rapid than those determined by simply setting $v \sim c$ in the expansion¹⁶⁻¹⁸. Relativistic expansions have had to be invoked to account for the observations of 3C120 and 3C273 which have had particularly rapid and intense fluctuations¹⁹. The dimensions that would be determined on the basis of a non-relativistic expansion would be so small as to entail high photon densities inside the source. Also, the inferred magnetic field would be very weak, so that a high electron density would be required to generate the observed synchrotron flux. These two effects together would lead to catastrophic inverse Compton scattering losses, so that the electrons would rapidly radiate their energy as X-rays or γ -rays instead of at radio frequencies. For the 1966-67 outburst in 3C273, $\Gamma \sim 3$ was required to avoid inverse Compton losses. Because the lifetime of relativistic electrons against synchrotron losses varies with the size of the source as R^5 , while the lifetime against inverse Compton losses varies as R^9 , it seems inevitable that at some early stage of the sources the inverse Compton losses must dominate. Kellermann and Pauliny-Toth have demonstrated²⁰ from the spectra of many radio sources with synchrotron self-absorbed components that there is a limit to their brightness temperature of 10^{11} to 10^{12} K. Because the brightness temperature at the peak frequency evolves as $T_b \propto R^{-(\gamma-1)/(\gamma+4)}$ the evolution is slow for typical values of γ and the observed limits on the brightness temperature seem to reflect the inverse Compton quenching early in the history of the source.

Only recently, by means of very long baseline interferometry²¹⁻²³, has it become feasible to achieve the angular resolution necessary to resolve these compact source components. In the VLBI technique, phase information is recorded locally at each observatory by reference to an atomic clock and the interference fringes are generated later by computer. The observations have been carried out at widely separated radio telescopes (Fig. 3 from ref. 23) and the resolution is ultimately limited by the diameter of the Earth. Observations at 6 cm and 18 cm over a Green Bank, West Virginia-Onsala, Sweden baseline have succeeded²⁴ in resolving components as small as $0''.001$ and placing limits on unresolved components as small as $0''.0006$. (It is interesting to note that the resolutions obtainable with the VLBI far exceed those of the most sophisticated "normal" optical observations. From Stratoscope observations Danielson *et al.*²⁷ have been able to place an upper limit of $0''.18$ on the non-thermal



Fig. 3. The radio telescope combinations that had been used for very long baseline interferometry up to mid-1968. Since then, other combinations over longer baselines have been successfully used (redrawn from ref. 23).

optical continuum component of the nucleus of the Seyfert galaxy NGC 4151. The optical intensity interferometer developed by Hanbury-Brown and his associates has attained resolutions of the order of $0''.0001$ (ref. 28), but it would probably be unable to pick out an individual component in an extended object; and in any case, no extragalactic objects are bright enough to be studied by this technique.) In several cases, multiple small components in one source were found. In addition to the $5'$ halo component²⁵ of the Seyfert galaxy 3C84, it was shown that there were two additional compact components $0''.03$ and $0''.0012$ in size. Their contribution to the integrated spectrum of 3C84 is indicated in Fig. 4, taken from the compilation of Kellermann and Pauliny-Toth²⁰. The smallest component is variable, and appears to be expanding at $\sim 0.1c$. Observations of 3C273 obtained over an Australian-United States baseline several times have supported the theoretical prediction that the component responsible for the 1966-67 outburst in this QSS expanded at a speed $v \geq 0.9c$, and have also yielded strong evidence for relativistic velocities in 3C279 (ref. 26). Some sources possess small components which are not known to be varying. There is a weak source at the centre of the galaxy M87 (Virgo A) which is less than three light months across²⁹.

Obviously the uniform, spherically, adiabatically expanding cloud discussed here grossly oversimplifies the actual situation within a radio source: indeed, one is surprised that such a model should fit the data adequately in even one case. It does, however, provide a generally valid estimate of the time-scale for the variability of a synchrotron source. The most significant conclusion that emerges from the observations so far is that the variations are never so rapid that they cannot be fitted by this type of model, with $\Gamma \lesssim 3$. (The sources comprise relativistic particles and magnetic fields, so bulk velocities $\sim c$ are dynamically plausible, and would indeed be expected unless the field is anchored to a large mass of non-relativistic material.) There would in principle be

no problem in specifying a suitably inhomogeneous or anisotropic model which could simulate the pattern of variations observed in any particular object. There is therefore no evidence that coherent emission mechanisms operate in extragalactic sources (as they must in flare stars or pulsars, where enormously high brightness temperatures are attained); nor is there yet any reason to doubt that all such sources are marginally resolvable by interferometers with baselines comparable with the Earth's diameter.

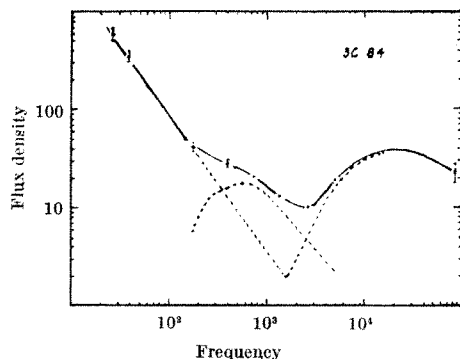


Fig. 4. The radio spectrum of the Seyfert galaxy NGC 1275 (3C84). The contributions of the two high frequency components are indicated by the dashed lines (from ref. 20). The flux density is in flux units ($1 \text{ f.u.} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$) and the frequency is in MHz.

Attempts to calculate the total energy involved in a single outburst on the basis of the simple expanding model yield values $\geq 10^{54}$ erg. This includes the magnetic field energy, the relativistic particle energy, and the bulk kinetic energy associated with the expansion. This estimate is extremely model dependent, but a lower bound can be inferred by integrating the radio power actually emitted over the duration of a burst. For the strong outburst observed in 3C273 this amounted to $\sim 10^{52}$ erg (assuming isotropic emission). According to Colgate³⁰, up to $\sim 10^{52}$ erg of relativistic particles can be produced by a single supernova. Thus it is tempting to identify each radio outburst with a stellar expansion, and to construe the observed radio variability as evidence that the "violent events" in galactic nuclear and quasar are due to multiple supernovae or stellar collisions. There is evidence^{31,32} that some of the bursts may require continuous acceleration of relativistic electrons over a period of a few months following their birth. As it seems likely that a rapidly spinning pulsar with stored rotational energy $\sim 10^{52}$ erg would remain at the centre of some supernova remnants, one may speculate that the required acceleration could be provided by a pulsar. (The rotational energy could be radiated by, for example, magnetic dipole emission over such a period.) A crucial observational test of this interpretation (and of others) would be to establish whether successive outbursts occur at slightly different positions. Typical dimensions for a dense galactic nucleus might be ~ 10 pc, corresponding to $\sim 0.03''$ at the distance of 3C120. Although this is much larger than the resolution of a VLBI, positions measured at different times cannot yet be correlated with such high accuracy.

The relationship between these small variable components and the large "dumb-bell" type sources which predominate in low frequency radio surveys remains an intriguing problem. A natural hypothesis is that the

extended components, involving total energies $\sim 10^{60}$ erg, represent the accumulated debris of many outbursts. The chief difficulty with this picture concerns the expected adiabatic losses associated with the vast expansion from dimensions ~ 1 pc to $\sim 10^5$ pc. Unless this can be counteracted (for example, by interactions with an external medium) the magnetic field strength, and the kinetic energy of electrons relative to the field, would decrease by such a large factor that the predicted radio emission from extended sources would fall far below what is observed. Piddington³³ has discussed an interesting possible alternative to the "multiple supernova" category of interpretations. He envisages that the central part of a galaxy may contain a differentially rotating gas cloud, in which the magnetic field would become more and more tightly wound. Eventually the magnetic pressure would cause the field to expand, like a spring, along the rotation axis. Piddington has suggested that double radio sources may originate in this manner. Furthermore, if coils of magnetic flux burst out of the disk one at a time, each may produce a "flare" which could be identified with an individual variable component.

Even if all the theoretical ideas discussed here have to be discarded in the light of further data, it seems evident that these variable radio components, often only a few light months across, are intimately linked to the primary energy source in the objects concerned, and that VLBI techniques will prove to be a powerful tool for investigating the enormously energetic and mysterious activity in quasar and galactic nuclei.

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Fertilization and Cleavage *in vitro* of Preovulator Human Oocytes

by

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Human oocytes have been taken from the mother before ovulation, fertilized *in vitro* and grown *in vitro* to the eight- or sixteen-celled stage in various media.

HUMAN oocytes removed from patients shortly before ovulation should assist the study of early human development¹. Previously, oocytes in dietyotene had been prepared for fertilization by maturing them in culture after their recovery from excised ovaries. In animals, fertilization of oocytes *in vitro* and *in vivo* after their maturation in culture results in embryos incapable of sustained growth; foetal development to full term is achieved by recovering oocytes just before ovulation, for example in metaphase of the first meiotic division, and completing their maturation *in vitro* before fertilization. We now present data on the fertilization and cleavage *in vitro* of human ova recovered by laparoscopy just before ovulation; preliminary observations have been given elsewhere^{2,3}.

Development beyond the pronuclear stage has proved difficult in several species, for example in mice. We have few human embryos for study, and have based our work on observations of animal embryos. Whittingham⁴ obtained viable young by fertilizing mouse ova *in vitro* with uterine spermatozoa, culturing the eggs to the 2-cell stage, and then transferring them into a recipient female. Whitten has shown⁵ that mouse ova recovered in their pronuclear stage can now be grown to blastocysts with complete success. The media used by Whittingham and Whitten were developed from earlier work^{6,7}. In addition to these media, we have also used Ham's F10 medium⁸ which has been used with rabbit embryos⁹, and Waymouth's medium MB752/1¹⁰ and medium 199¹¹ which are both widely used in tissue culture.

Oocytes and Spermatozoa

Patients were given injections of human menopausal gonadotrophin and chorionic gonadotrophin to induce follicular growth and maturation. Laparoscopy was performed 30–32 h after the injection of HCG, and each follicle was aspirated separately¹. The oocytes were suspended in droplets consisting of fluid from their own follicle (where available), and the medium being tested for fertilization. After incubation for 1–4 h at 37° C the oocytes were washed through two changes of the medium under test before being placed in the suspensions of spermatozoa. Preovulatory oocytes would be ready for fertilization, that is, in metaphase of the second meiotic division, by 3–4 h after collection. Many oocytes were obviously not preovulatory, and therefore unsuitable for fertilization, but all were placed in the fertilization droplets in order to simplify our procedure.

Ejaculated spermatozoa were supplied by the husband. The spermatozoa were washed twice by gentle centrifugation in the medium under test, and made up to a final concentration of between 8×10^5 and 2×10^6 /ml. depending on the quality of the sample. The higher numbers were used

with samples of poor quality containing many inactive spermatozoa, cellular inclusions, other debris, or viscous seminal fluid. The fertilization droplets were approximately 0.05 ml.

Oocytes classified as preovulatory were surrounded by layers of silvery-appearing corona and cumulus cells in a viscous matrix¹. Oocytes classified as non-ovulatory were enclosed in a few layers of corona cells. Atretic oocytes had few or no cells. Initially, each oocyte was placed in its own fertilizing droplet, but later all the oocytes recovered from a patient were grouped together. The mass of cells surrounding preovulatory oocytes led inevitably to some dilution of the numbers of spermatozoa in the fertilization droplet.

Fertilization

Bavister's medium had been capable of sustaining fertilization in previous work^{12–14}, and was therefore used extensively. This medium was slightly modified during the work by reducing the sodium chloride to 0.75 g per cent and increasing the KCl to 0.039 g per cent, following the analysis of the amount of Na⁺ and K⁺ in human follicular fluid. Whittingham's medium was used unmodified, and both Waymouth's medium (Flow Laboratories) and Ham's F10 (Flow Laboratories) were modified by raising the pH to 7.5–7.6 with extra bicarbonate and supplementing with bovine serum albumin (0.36 g per cent). Sodium pyruvate was also added to Waymouth's medium (1.1 mg per cent). The gas phase was either 5 per cent CO₂ in air, or 5 per cent CO₂, 5 per cent O₂ and 90 per cent N₂.

The cumulus cells began to dissociate within 2–3 h of insemination, although corona cells remaining closely or loosely attached prevented the examination of some oocytes by low-power microscopy 15 h after insemination. The cells were left in place after attempts to remove them had led to damage of some oocytes. Criteria for judging fertilization were: (i) the observation by phase-contrast microscopy of two pronuclei, two polar bodies and, if possible, a sperm tail in the cytoplasm of the oocyte; (ii) identification of pronuclei in the ova in culture, using a stereoscopic low-power microscope (this method was inexact, and even impossible when cells persisted around the oocyte, but the eggs remained relatively undisturbed for further development); (iii) cleavage of the egg, preferably after the identification of pronuclei.

Data on the incidence of fertilization are given in Table 1. Only a few ova could be spared for examination by phase-contrast microscopy. Fertilization was observed in Bavister's medium, modified Waymouth's medium and in Whittingham's medium, but not in modified Ham's F10 on the sole occasion it was used. Low rates of fertilization sometimes seemed to be a consequence of the poor quality of the spermatozoa from some patients.

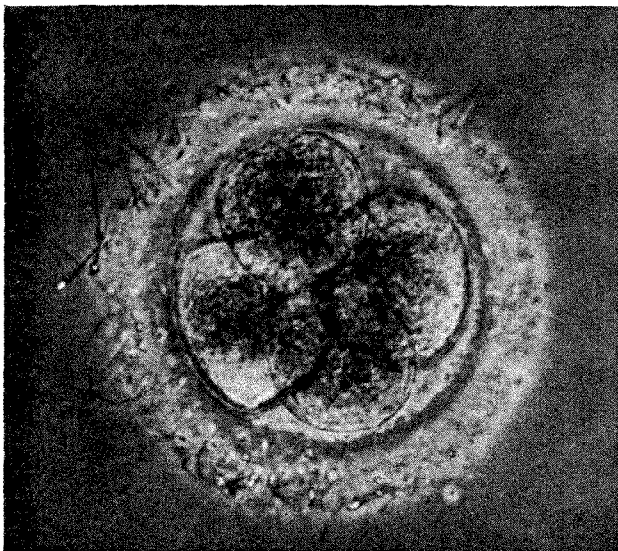


Fig. 1. A 4-celled egg grown in Waymouth's medium supplemented with calf serum. Development of this embryo had evidently been arrested.

Cleavage

Between 12 and 15 h after insemination, the oocytes were gradually transferred from the medium used for fertilization into various other media for cleavage. At least five embryos were cultured in each medium, except for medium 199. All media were adjusted to a pH of approximately 7.3. A total of thirty-eight embryos cleaved in culture (Table 2). Two pronuclei had been observed in many of them. Almost all eggs cleaved twice, and a few completed their fourth cleavage.

Many ova cleaved regularly and evenly in Whittingham's medium, and most reached the 8-celled stage. One embryo

behaved anomalously, for it divided into two cells and then reverted to one cell.

Whitten's medium is similar to Whittingham's except for having a lower osmotic pressure. Development became distorted when embryos were cultured in this medium. Cleavage became irregular, a 2-celled egg reverted to one cell, and cytoplasmic division seemed to occur without nuclear division in three embryos. For example, one embryo cleaved very rapidly from 3 cells to an apparently normal 8-celled embryo, but when flattened and stained¹⁵ was found to have only three nuclei. When the osmotic pressure of this medium was increased by adding more Na⁺ and K⁺ ions, cleavage became more regular although slower than in other media.

Waymouth's medium supplemented with 15 per cent v/v foetal calf serum failed to support development from the 8-celled stage, perhaps because of the high osmotic pressure: ova placed in it showed retractions of the cell surface. One embryo cultured in medium 199 became arrested in the 4-cell stage.

Ham's F10 supplemented with 20 per cent foetal calf serum was used at two osmotic pressures by adjusting the amount of water added: at 287 milliosmols/Kg cleavage appeared abnormal in two embryos. At 300-305 milliosmols/Kg seven embryos showed excellent cleavage to a stage approaching or beyond the 16-cell stage. Another embryo photographed in the 8-celled stage (Fig. 2) would probably have cleaved further.

The embryos were inspected at various times during culture. The first cleavage occurred before 38 h post-insemination. The second cleavage occurred between 38 and 46½ h in seven embryos cultured in Whittingham's medium or Ham's F10, and the third cleavage between 51 and 62 h in four embryos. The fourth cleavage occurred before 85 h in embryos grown in Ham's F10. Timings of cleavage in other embryos did not differ from these estimates, but the intervals between recordings were too long for accurate estimates.

The embryos were left in culture until it was clear that development had ceased, that is, approximately 48 h after

Table 1. INCIDENCE OF FERTILIZATION *in vitro*

Medium	O ₂ in gas phase	Total No. of oocytes	Non-ovulatory	Phase-contrast microscopy Unfertilized	Fertilization				
					Fertilized	Others	1-celled	Low-power microscopy Pronucleate and cleaved	Pronuclei not seen, egg cleaved
Bavister's	20 per cent	105	30	26	3+1*+1?	9	18	11†+2?‡	4
	5 per cent	76	—	—	—	—	53§	6+2?‡	15
	Combined	181	30	26	3+1*+1?	9	71§	17†+4?‡	19
Whittingham's	20 per cent	19	3	5	—	3§	5§	2	1
Modified Waymouth's	20 per cent	10	2	1	2	2	1	1	1?
Modified Ham's F10	5 per cent	2	—	—	—	—	2	—	—

* Sperm in perivitelline space.

† Four of these eggs were transferred while pronucleate into the oviduct of a rabbit.

‡ Probably pronucleate.

§ One egg in each of these groups was found to possess two or more pronuclei between 48 and 72 h after insemination, and might have been undergoing rudimentary parthenogenetic development.

Table 2. CLEAVAGE OF THE EMBRYOS *in vitro*

Medium	Osmolality (mosm/Kg)	O ₂ in gas phase (per cent)	Total No. embryos	Final No. of cells in the embryos							
				1	2	3	4	4-8	8	8-16	16 or more
Whittingham's	280-290	20	9	1*	—	—	1	2	4	1	—
	280-290	5	2	—	—	—	—	—	2	—	—
Whitten's	270	5	5	1*	—	1	—	—	1	2†	—
	325	5	3	—	—	—	—	—	2†	1	—
	325	20	6	1‡	—	—	2	2	1	—	—
Waymouth's with 15 per cent inactivated foetal calf serum	292	20	1	—	—	—	1	—	—	—	—
199 with 20 per cent inactivated foetal calf serum	287	5	2	—	—	2	—	—	—	—	—
Ham's F10 with 20 per cent inactivated foetal calf serum	300-305	5	10§	—	—	—	—	1¶	1**	4	3

* Reverted to 1-cell after initial cleavage.

† Both embryos had cells of dissimilar size, and cleaved erratically.

‡ Probably reverted to 1-cell after cleavage, but vision obscured by corona cells.

§ One embryo became infected at 2-cell stage.

¶ Cleavage erratic.

**Removed from culture and photographed in 8-cell stage.

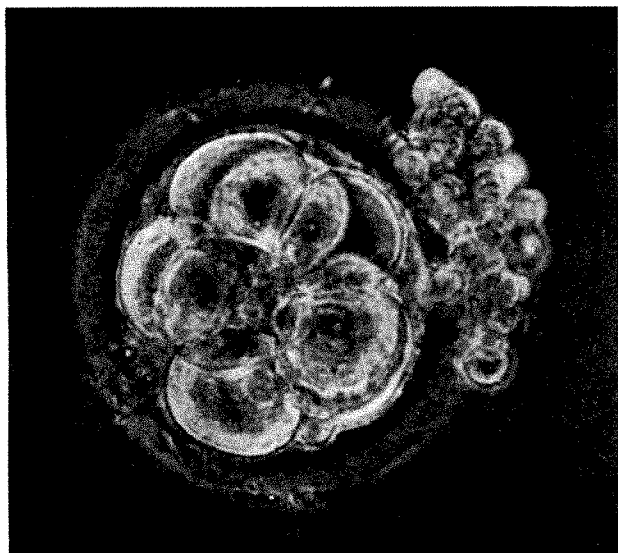


Fig. 2. An 8-celled egg grown in Ham's F10 supplemented with calf serum. It was removed from culture during cleavage, and could have been capable of further development.

the previous recording of cell division. Many embryos were now displaying fragmentation of blastomeres or other forms of degeneration. The blastomeres of many embryos that had cleaved normally possessed a single nucleus as judged by phase-contrast microscopy. Whole mounts or flattened preparations of two 16-celled, three 8-celled, and two 4-5-celled embryos revealed the same number of nuclei as cells in each embryo. One of the 16-celled embryos possessed mitoses. One embryo classified while living as 16-celled possessed twenty-one nuclei, although some were smaller than others.

Comment

The amount of free follicular fluid added to the fertilization droplets must have been small, except for that in the viscous cumulus masses surrounding the oocyte. Various factors in follicular fluid are believed to be necessary for stimulating the spermatozoa and inducing changes in the acrosome¹⁶, and steroids present in follicular fluid or synthesized by the granulosa cells could also be involved. Recent studies have indicated that the acrosome is a modified lysosome¹⁷, and capacitation could involve agents known to destabilize lysosomal membranes. Among the most potent of these agents are progesterone and other progestogens¹⁸. Progesterone has been identified in human follicular fluid aspirated with the oocytes (unpublished work of K. Fotherby and of ourselves), and granulosa cells cultured *in vitro* can also synthesize progesterone and other progestogens¹⁹. Ultrastructural examination has revealed the acrosomal changes following capacitation are seen as the spermatozoa penetrate between the cumulus cells²⁰. We have measured the levels of oestradiol-17 β and of LH in many of these fluids²¹, and are currently measuring progesterone and 17 α -hydroxyprogesterone (unpublished work of K. Fotherby and of ourselves). These fluids could then be tested for their efficacy in inducing the acrosome change in spermatozoa in relation to the known levels of the different steroids. It would seem to be a wise precaution to remove the cells surrounding the oocytes before testing agents inducing capacitation and fertilization *in vitro*.

Cleavage occurred in all ova seen to possess pronuclei, and in some others that could not be examined because of the enveloping cells. Human embryos may have metabolic needs similar to mouse embryos, as judged by their cleavage in simple defined media. Firm conclusions about the value of different media obviously cannot be drawn when so few ova were available. On occasions,

four or five embryos were obtained from one patient, and comparisons could be made between different media. Results with defined media were inferior to those obtained from Ham's F10 supplemented with foetal calf serum and with an osmotic pressure of around 300 osmols. Among various differences, F10 contains salts of heavy metals and more pyruvate than the other media. Development beyond the 8 or 16-cell stage might demand the presence of human oviductal or uterine secretions, and these could be collected with a small chamber placed in the uterus for a few hours²². The premature recovery of preovulatory oocytes might be another cause of the arrested development, and they might have to be aspirated even closer to the time of ovulation.

Analysis of the chromosome complement of the embryos can be undertaken now that some cleavages have been timed, although perhaps better delayed until later stages of development are available. Identification of a Y chromosome in some embryos is now critically needed to furnish formal proof to confirm the morphological evidence of fertilization used currently. The quinacrine dyes might detect the Y chromosome in interphase nuclei and mitoses, as they do in somatic cells^{23,24}, although caution will be necessary, for they fail to stain the Y in most spermatogonia²⁵. Chromosomal analysis of the embryos should also reveal anomalies in early development, for example, non-disjunction and especially triploidy arising through polyspermy or failure of polar body formation. When more preovulatory oocytes are available their parthenogenetic activation might be used to produce haploid strains of cells, as in mice²⁶.

One or more embryos have been produced from twenty-nine of the forty-nine patients under treatment in this work. The normality of embryonic development and the efficiency of embryo transfer cannot yet be assessed, although conditions for implantation in the treated patients should be favourable¹.

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Model for DNA Replication by Kornberg's DNA Polymerase

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Experimental evidence is given for a model in which DNA replicates with the two growing daughter strands covalently linked by a pyrophosphate bridge. The two strands are synthesized synchronously in opposite directions, one 5' to 3' and the other 3' to 5'.

THE elegant studies of Kornberg and his collaborators have shown that *Escherichia coli* DNA polymerase has a remarkable multiplicity of functions¹. A basic feature of the catalytic properties of this enzyme is that its polymerization of deoxyribonucleoside triphosphates into DNA proceeds in the 5' to 3' direction. Because the two strands of the DNA double helix are antiparallel, a fundamental problem of DNA replication is to explain how synthesis occurs also in the 3' to 5' direction. We propose a model in which the two new strands of replicating DNA are synthesized together because they are covalently joined by a pyrophosphate bridge. Recent work has suggested that DNA polymerase may not replicate DNA *in vivo*, but may rather be a repair enzyme². Our model shows how the Kornberg enzyme may be capable of catalysing both the replication and the repair of DNA.

Renaturability of Replicating DNA

One essential prediction of our model is that the newly synthesized DNA should be renaturable*. Three lines of evidence for believing this to be so are:

Kornberg's group³ found that the product of replication of T7 and *Bacillus subtilis* DNA using fraction IX DNA polymerase was renaturable. Electron micrographs showed the product to be highly branched, and Kornberg proposed that the polymerase could switch from one parental template strand to the other. This would allow 5' to 3' synthesis at all times and would give rise to renaturable DNA.

Replication⁴ of the synthetic DNA, d(T-G)_n·d(C-A)_n, with highly purified DNA polymerase results in the production of a DNA of hybrid density in alkaline caesium chloride gradients, indicating that the two complementary strands are no longer separable. Paetkau has shown that in the presence of less highly purified DNA polymerase no hybrid DNA was obtained after many-fold replication and he suggested that the crude polymerase contained a factor which specifically broke the covalent attachment between the d(T-G)_n and d(C-A)_n strands.

Pauling and Hamm⁵ have recently shown that a significant fraction of *E. coli* DNA replicated during a short pulse is renaturable. This suggests that renaturable DNA is not an artefact of replication *in vitro*, but is an essential feature of DNA replication *in vivo* as well.

Both the Kornberg model and our model are in agreement regarding the formation of renaturable DNA. The Kornberg model proposes, however, that one parental strand is displaced from the duplex during copying by DNA polymerase, and such a model is inconsistent with the following observations made in this laboratory.

With Khorana polymers⁶ such as d(T-G)_n·d(C-A)_n one can attempt to replicate just one strand by adding only two dNTPs, for example dATP and dCTP, in order to synthesize d(C-A)_n. But in these conditions there was neglig-

ible synthesis of d(C-A)_n. As a control, on the addition of all four dNTPs there was many fold net synthesis of d(T-G)_n·d(C-A)_n. Further, single-stranded d(T-G)_n with short primers of d(C-A)_n did give rise to one-fold synthesis of d(C-A)_n on d(T-G)_n when only dATP and dCTP were present, after which synthesis abruptly stopped. Thus in repair-like synthesis DNA polymerase is very active even with only two dNTPs. d(C-A)_n primers did not, however, stimulate d(C-A)_n synthesis by duplex d(T-G)_n·d(C-A)_n, which suggests that strand displacement does not occur on a duplex DNA, and that all four NTPs are required for replication. Also consistent with this interpretation is the finding of Wells *et al.*⁷ that *in vitro* synthesis of various synthetic polymers leads to identical synthesis of each strand.

Very short pulse-labelling of *in vitro* replicating natural DNA showed the labelled product to be almost totally renaturable. In a strand displacement model the newly synthesized DNA should be denaturable—at least before strand switching occurs.

These data can be explained, however, if during the replication of DNA, the two daughter strands are covalently linked. Moreover, certain predictions can be made on the basis of this model. Some of these have been tested, and the data are consistent with the proposed mechanism.

Model for DNA Replication

The essential features of this model are: (1) The parental DNA divides at the replicating point where both daughter

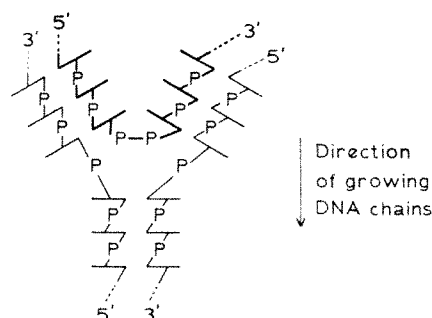


Fig. 1. The replicating fork of DNA. The daughter strands are shown linked by a 3' to 5' pyrophosphate diester, forming one molecule.

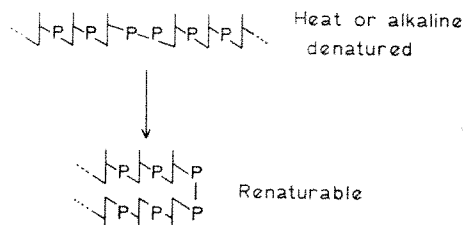


Fig. 2. The daughter strands of replicating DNA shown in their denatured state and on removal of denaturing conditions.

* Renaturable DNA is defined as DNA that after denaturation (by heat or alkali, for sample) rapidly reverts to a duplex when the denaturing conditions are removed. This term is preferred to non-denaturable⁸, for the DNA is denatured in the denaturing conditions.

strands are laid down synchronously in a Y type structure (Fig. 1) as originally proposed by Watson and Crick and as suggested by Cairns's autoradiogram⁸. (2) The two daughter strands are covalently joined by a 3' to 5' pyrophosphate bridge (Fig. 1), which makes the newly synthesized DNA renaturable (Fig. 2). (3) Two complementary deoxyribonucleoside triphosphates (dNTPs), with their bases paired in the Watson-Crick manner, are incorporated simultaneously at the replication site. (4) A concerted rearrangement takes place (Fig. 3) to incorporate a nucleotide unit into each of both daughter strands with the elimination of two pyrophosphates but retention of the pyrophosphate bridge.

Fig. 1 depicts the 3' to 5' pyrophosphate diester link between the two daughter strands of DNA at the replicating point. Because the two daughter strands synthesized by one parental molecule are complementary, upon heat or alkaline denaturation, they will spontaneously rewind into a duplex starting at the pyrophosphate link (Fig. 2). The chemistry of chain elongation is illustrated in Fig. 3. The parental DNA has been omitted for clarity and only the essential features of bond breakage and formation have been included. On the left, chain elongation is shown proceeding in the 5' to 3' direction. Attack of some basic function (here shown as the oxygen on the γ -phosphate but a basic group on the enzyme is equally likely) on the 3'-OH of the incoming triphosphate leads to the concerted reaction shown. The overall result is that, first, the phosphorus marked with an asterisk is transferred to the incoming nucleotide unit and remains at the replicating point, and second, a new internucleotide bond is formed with the elimination of pyrophosphate. On the chain shown on the right, growth takes place in the 3' to 5' direction by a very similar concerted reaction but in which the phosphate in the link is retained to form the new

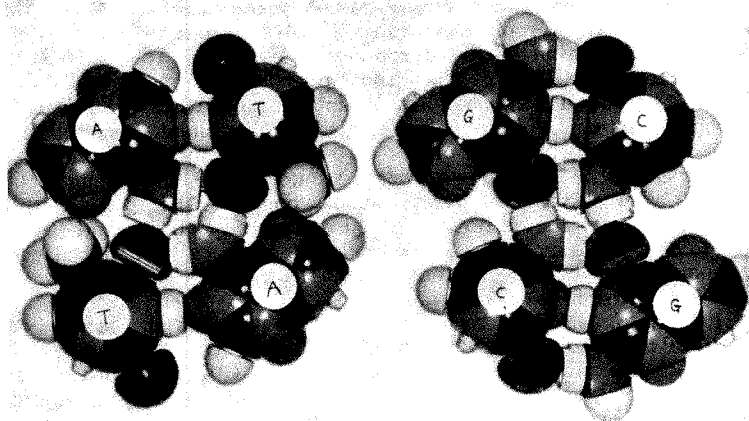


Fig. 4. The CPK model of the specific hydrogen-bonded arrangement for recognition of parental base pairs in DNA by incoming base-paired triphosphates, as predicted by the model of Kubitschek and Henderson.

phosphodiester bond. In this way the α -phosphate of the triphosphate always forms the phosphodiester backbone, as it must if it is to be consistent with the known chemistry of nearest neighbour analysis. Clearly both daughter strands must grow synchronously if DNA replicates by this mechanism. The net result of the two concerted reactions is that both daughter strands have grown by one nucleotide unit, one in the 5' to 3', and the other in the 3' to 5' direction. At all times they have been covalently held together and one phosphate, distinguished by the asterisk, is unique in that it has been transferred to an incoming nucleotide and conserved at the growing point.

Fig. 3 shows that a P-O grouping must span the 3' oxygen and the α phosphorus of the incoming deoxyribose triphosphate for both 5' to 3' and 3' to 5' synthesis. CPK model-building shows that there are several possible conformations with excellent contacts which would lead to compact structures suitable for the bond breakage and formation depicted in Fig. 3. The stereochemical relationship between the parental and daughter strands is based on the master strand model for DNA replication proposed by Kubitschek and Henderson⁹, which predicts—on the basis of genetic evidence—that base-paired triphosphates are incorporated at the replication site. Fig. 4 shows a CPK model illustrating the recognition of a parental base pair by an incoming base pair (thus providing template specificity). To obtain isomorphous structures, only A-T base pairs recognize T-A base pairs, and only G-C base pairs recognize C-G base pairs with the specific hydrogen-bonding patterns shown. An attractive feature of the model is that some of the new hydrogen bonds between parental and daughter DNA are made before separating the parental base pairs, and this suggests a source of energy for breaking the parental base pairs. The specificity for DNA replication lies intrinsically in the stereochemistry of the bases, and it would not be necessary for the enzyme to "feel out" two bases, by some allosteric mechanism, nor would the strands of the parental DNA have to be peeled apart before replication. The CPK model of the replicating site shows that the mechanism of Fig. 3 is stereochemically feasible, with the hydrogen bond between the parental base pair breaking before the actual incorporation of the new nucleotides.

Evidence for the Model

The model predicts, first, that newly synthesized DNA should be renaturable at all times. Any DNA synthesized by a strand-switching mechanism^{3,10} would, during the initial phase of its synthesis at least, be denaturable. Second, any treatment which ruptures a pyrophosphate

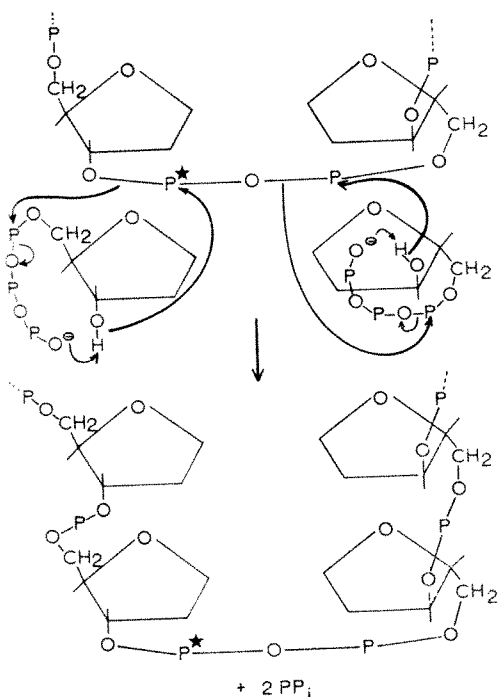


Fig. 3. The mechanism for the incorporation of base-paired deoxyribonucleoside triphosphates into DNA with chain elongation in the 5' to 3' and 3' to 5' directions.

diester should make renaturable DNA denaturable. Third, initiation of replication must involve a unique event—the formation of the pyrophosphate bridge. If it is assumed that P* in Fig. 3 is the β phosphate of one of the base-paired triphosphates initiating replication, then synthesis of DNA from β,γ -labelled dNTPs should result in the incorporation of ^{32}P into DNA. Fourth, this ^{32}P should be resistant to phosphomonoesterase but sensitive after cleavage of pyrophosphate bonds. Fifth, the β,γ - ^{32}P counts should not be chased out by unlabelled dNTP once synthesis has been initiated.

Experimental evidence, albeit incomplete, tends to support the first four of these predictions.

(1) Using *E. coli* DNA as a template > 80 per cent of ^{14}C -dGTP incorporated *in vitro* during a 30 s pulse, carried out after preliminary repair synthesis at 20°C ¹¹, was insensitive to exonuclease I treatment (specific for single-stranded DNA) even after alkali or heat denaturation of the newly synthesized DNA.

(2) Using P_1P_2 -dithymidine 5'-pyrophosphate, $(-\text{pT})_2$, as a model substrate, we have developed two methods for specifically cleaving pyrophosphate diesters. $(-\text{pT})_2$ was converted quantitatively to pT by appropriate incubation in pyridine containing 2 per cent water or in 0.1 M glycine buffer (pH 10) containing 0.01 M LaCl_3 . Phosphodiester bonds must be quite stable in these conditions, for the sedimentation coefficient of λ DNA in alkali ($s_{20,w} = 31.8$ in 0.1 M NaOH, 0.9 M NaCl, 10^{-3} M EDTA) was unaffected by these treatments. Using the pyridine-water treatment, renaturable DNA was converted to a denaturable form, as determined by alkaline denaturation and exonuclease I treatment. The DNA, after denaturation, was degraded to the extent of 40–50 per cent by exonuclease I. The model would predict this, because one-half of the denatured DNA should terminate in a 3'-phosphate, and exonuclease I requires a free 3'-hydroxyl to initiate degradation of single-stranded DNA¹².

(3) All four β,γ - ^{32}P -labelled dNTPs were obtained by DNA polymerase catalysed pyrophosphate exchange of dNTPs¹³, one at a time, a very high specific activity being necessary to detect the one ^{32}P at the growing point. To isolate DNA without a contaminating background, the reactions were terminated by adding a ten-fold excess of EDTA over Mg^{2+} , and SDS to 0.04 per cent followed by gel filtration on 'Bio-Gel' agarose (15 M) in 0.05 M triethylammonium bicarbonate. In reactions containing all four dNTPs (β,γ -labelled), DNA isolated as above was found to be labelled with ^{32}P . The DNA was heated at 70°C with dNTPs, PP_i and 0.04 per cent SDS and after gel filtration all the ^{32}P was quantitatively recovered with the DNA, ruling out artefacts resulting from binding of labelled dNTPs or PP_i . In addition, reaction conditions which should give largely repair synthesis of DNA¹⁴ gave no detectable ^{32}P in the DNA, showing that no label was in the α position of the dNTPs. The ^{32}P in the DNA was insensitive to bacterial alkaline phosphatase at 70°C ¹⁵, as predicted, and this shows that the label is not present as a 5'-triphosphate at the end of the DNA.

(4) After treatment of ^{32}P DNA with pyridine-water to rupture pyrophosphate diesters, the ^{32}P became sensitive to alkaline phosphatase.

Replication and Repair *in vivo*

The evidence which might argue against the validity of the model is: first, the recent isolation by De Lucia and Cairns² of a mutant of *E. coli* which seems to lack the Kornberg polymerase. As the authors point out, however, their data do not exclude the possibility that the Kornberg enzyme plays a part in normal DNA replication. In fact, the low residual level of activity suggests that there may be five to ten molecules of polymerase per cell and presumably only one or two are necessary for DNA replication. In wild type bacteria each cell contains several hundred molecules of polymerase and their main function may be repair of DNA lesions, which would be con-

sistent with Kornberg's findings¹⁶ and the ultraviolet sensitivity of the mutant².

Second, the production of "short" pieces of DNA by pulse-labelling *in vivo*¹⁷ is consistent with models for replication in which synthesis always occurs in the 5' to 3' direction. Because at least one daughter strand would have to be synthesized discontinuously, it has been suggested that polynucleotide ligase must play a part in DNA replication^{18,19}. But recent experiments by Kozinski and his colleagues^{20,21} strongly suggest that the ligase is not necessary for DNA replication, but is required for repairing endonucleolytic nicks. Because DNA seems to replicate in large membranous structures^{22–25}—and membranes are rich in nucleases²⁶—the newly synthesized DNA may perhaps be vulnerable to nucleases when the cell is disrupted. The short fragments of newly replicated DNA of bacteriophage SPP-1²⁷ hybridize equally well to either complementary strand of parental DNA, which would not be predicted on the simplest strand displacement model³ for one daughter strand should be continuously elongated.

Third, the preferential release by exonucleases of ^{14}C or ^3H counts from DNA uniformly labelled with ^{14}C and labelled at the growing ends with ^3H has been used to demonstrate synthesis in the 5' to 3' direction²⁸. The ^3H pulse was at 8°C , however, which is favourable for repair but not for replication¹¹.

Published evidence which seems to support this model for DNA replication includes the following. First, although Cairns concluded from his autoradiographic studies that the replicating region had a Y shaped configuration, the resolution was not sufficiently high to permit any deductions regarding the molecular anatomy of that region. But electron micrographs of replicating DNA suggest that there is little, if any, region of single-stranded DNA at the replicating point in the DNA of such diverse organisms as polyoma virus²⁹, λ phage³¹, *E. coli*, mycoplasma³² and cultured mammalian cells (L5178Y)³³. In the case of replicating L5178Y cells, the resolution was 10 Å.

Second, Kubitschek and Henderson's model of a master strand for DNA replication is based on the absence of segregation of mutations in heterozygotes (mismatched base pairs)³⁴. It is very difficult to conceive of any mechanism of DNA replication that could account for this observation unless base-paired triphosphates are the "unit" of incorporation in polymerization. Their model has the added attraction of being able to rationalize the observed very low mutagenic rate *in vivo*. Evidence has recently been presented, however, for segregation of heterozygotes, or their destruction, on replication³⁵. If the replication enzyme carries out the repair function as well, which we are proposing, the latter explanation is more likely.

Third, the two exonuclease activities associated with highly purified DNA polymerase^{36,37} which degrade from the 5' and 3' ends make it difficult to reconcile the various activities of the polymerase¹⁴. In our model, however, there is no free 3' or 5' end in the replicating region of the DNA and this commits the polymerase to replication.

Fourth, multiple lengths of genomes³⁸ could readily arise by replication of circular DNA, with a duplex DNA as the product. The rolling circle model for DNA replication³⁹ which envisages single-strand displacement from a DNA duplex, also predicts multiple lengths of genomes. The synthesis of single-stranded DNA from a double-stranded DNA template, however, is probably unique to those bacteriophage systems (for example ϕX 174) in which the genome of the mature phage is itself single-stranded. Moreover, such specialized replication probably requires the intervention of factors for which the viral genome codes⁴⁰. Finally, oligonucleotides with 5' triphosphate ends are not elongated in the 3' to 5' direction²⁸, which is consistent with our model.

In conclusion, the model presented here is teleologically satisfying, and is supported by our preliminary experimental evidence. In addition it raises certain intriguing questions with regard to the initiation and termination of

replication. At the moment I favour the idea of an initiation factor which would be present in fraction IV of the Kornberg polymerase preparation. We can obtain routinely 70-fold replication of $d(TG)_n-d(CA)_n$ and other synthetic polymers in the presence of fraction IV and pure polymerase. It seems unlikely that this is entirely the result of endonucleolytic nicks, especially because such nicks do not give rise to net-fold synthesis¹⁴. The other alternative, that fraction IV greatly increases the slippage rate, is not appealing.

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Note added in proof. Recently, experiments designed to label DNA using β , γ -³²P-labelled dNTPs as substrates yielded ³²P-labelled material that was excluded off agarose (15 M), was insoluble in TCA and resistant to alkaline, but did not band with DNA in a CsCl gradient. This throws some of the above interpretations into question. Although these results make a pyrophosphate link less likely, however, the basic principle of the model, namely, concomitant synthesis off both strands of DNA with daughter strands covalently linked, is still attractive, for modifications of the above model are readily constructed.

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T4 Bacteriophage Gene 32: A Structural Protein in the Replication and Recombination of DNA

by

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A new type of protein essential for DNA replication and genetic recombination has been isolated from T4 bacteriophage-infected cells of *E. coli*. This protein binds cooperatively to single-stranded DNA, and it catalyses DNA denaturation and renaturation in physiological conditions *in vitro*.

GENETIC recombination involves the precise breakage and reunion of "mating" double-stranded DNA molecules at points of mutual sequence homology¹⁻³. Recombinant DNA molecules have been shown to contain a heterozygous region, which seems to be formed during the fundamental event in the recombination process^{2,4}. Although the actual mechanism of genetic recombination is unknown, several relatively simple models have been proposed⁵⁻⁷; these assume an unusual fluidity of DNA structure within the cell which allows efficient testing for complementary base pairings between strands of randomly colliding DNA molecules. For example, in the scheme

proposed by Holliday⁵, local DNA denaturation is invoked to open mating DNA helices at homologous regions, followed by DNA renaturation between single strands thereby exposed on opposite molecules. *In vitro*, however, the DNA double-helix is overwhelmingly stable relative to the single strands in physiological conditions⁸, and locally denatured regions more than a few base-pairs long should consequently occur only very rarely. This expectation is borne out by experimental studies of the stability of the short helix formed by the "cohesive ends" of isolated bacteriophage lambda DNA, for which, even in low [Na⁺] (0.033 M), transient melting of twelve contiguous base-

pairs occurs with a relaxation time of about 7 days at 37° C (ref. 9). By contrast, at 37° C within the cell, the average T4 DNA molecule participates in more than one recombination exchange every 10 min¹⁰, whereas it must unwind completely during each round of DNA replication.

Single-stranded regions of DNA postulated as intermediates in various models for the recombination process could easily be generated by the action of exonuclease^{7,11,12} or DNA polymerase⁶ within the cell, if not by denaturation. It might be expected that the complementary single-stranded regions so generated would rapidly pair by renaturation because of the overwhelming stability of the double-helical conformation at 37° C. Single-stranded DNA folds on itself, however, to create imperfectly hydrogen-bonded, intra-strand helices in physiological conditions *in vitro*¹³. These folds make the DNA bases relatively inaccessible, and thereby prevent complementary single strands from finding satisfactory pairings^{14,15}. As a result, raising the temperature from 37° C to 68° C increases renaturation rates as much as 1,000-fold. This is purely a kinetic effect, for the equilibrium stability of the double-helix relative to single strands is greater at the lower temperature.

We have discovered a DNA-binding protein in the T4 bacteriophage system the properties of which suggest a solution to this problem of DNA mechanics. The protein is the product of T4 gene 32. The "32-protein" is required for the genetic recombination of T4 bacteriophage DNA¹⁶; in addition, it is one of several gene products known to be essential for T4 DNA replication¹⁷.

Biological Role of T4 Gene 32

The product of T4 gene 32 is required for T4 DNA replication throughout the infectious cycle; an amber mutant in this gene requires 40 min at 37° C to approximate even a single round of replication¹⁸, whereas temperature-sensitive mutants which are allowed to begin synthesizing DNA at 25° C stop replication when shifted to a non-permissive temperature^{19,20}. (For one particular mutant, *ts* P7, all replication ceases within 1 min after a shift to 42° C: S. Riva, A. Cascino, and E. P. Geiduschek, manuscript submitted for publication; unpublished results of M. Curtis and B. M. A.) Moreover, gene dosage experiments show that gene 32 is unique among the T4 genes known to affect DNA metabolism in that its product is required stoichiometrically rather than catalytically; that is, as for structural proteins of the phage particle, the quantity of 32-protein synthesized in the infected cell directly limits the number of progeny phage produced²¹. This finding must be reconciled with the fact that about 10,000 molecules of 32-protein are made in a normal infection, and very few, if any, are used up in the construction of mature phage particles²⁰. It therefore seems that 32-protein plays a structural part in the replication of T4 DNA.

Another important biological observation concerning gene 32 is that, as first shown by Tomizawa and co-workers, its function is necessary for the formation of the hydrogen-bonded joint DNA molecules believed to be the initial products of genetic recombination^{2,16,18}. In this connexion, it should be noted that DNA replication does not seem to be required for recombination of T4 DNA¹⁸, and that recombination-deficient mutants in another bacteriophage system (phage λ) replicate their DNA normally¹¹. It is therefore likely that 32-protein functions directly in both of these genetic processes.

Properties of Purified 32-Protein and its Binding to DNA

We have previously reported that at least twenty different DNA-binding proteins are synthesized after T4 bacteriophage infection of *E. coli*, as judged by DNA-cellulose chromatography²⁰. One of the principal DNA-

binding proteins was identified as the product of T4 gene 32, for it is altered after infection with bacteriophages carrying amber and temperature-sensitive mutations in this gene^{19,20}. The 32-protein is made in large quantities at both early and late times of infection, about 10,000 molecules accumulating per infected cell.

In the absence of a direct assay for 32-protein, the course of its purification was originally monitored by polyacrylamide gel electrophoresis. In the work to be described stepwise elution from a single-stranded DNA-cellulose column followed by DEAE-cellulose chromatography has been used to prepare 32-protein which is electrophoretically homogeneous.

In spite of its tight binding to polyanionic DNA, 32-protein carries a net negative charge at pH 7. As estimated from a combination of sedimentation and gel filtration data, the molecular weight of the native protein is 35,000, and the axial ratio for an equivalent prolate ellipsoid is about 4 (ref. 19). Because the same molecular weight is obtained for denatured, reduced 32-protein in sodium dodecyl sulphate (SDS)-containing polyacrylamide gels²², the native protein seems to consist of a single polypeptide chain²⁰.

Purified 32-protein binds strongly to single-stranded DNA, as seen by the co-sedimentation of ³H-leucine-labelled protein with such DNA through stabilizing sucrose gradients. The affinity of 32-protein for DNA decreases gradually as the salt concentration is increased from 0.15 to 0.60 M, suggesting the importance of electrostatic forces in the binding. It seems likely, therefore, that the region of polypeptide chain in direct contact with the DNA includes a concentration of positively charged residues spaced so as to interact with the DNA phosphates, even though the protein as a whole carries a net negative charge.

The stoichiometry of the tight complex which 32-protein forms with single-stranded DNA at low salt concentrations has been examined by sucrose gradient sedimentation of a fixed quantity of labelled 32-protein in the presence of varying amounts of the circular, single-stranded DNA from bacteriophage fd²³. At lower concentrations of DNA, two distinct peaks of radioactive protein are seen; one sediments rapidly with the DNA, the second at the slow rate characteristic of the free protein. The free protein peak is absent above a weight ratio of DNA to protein of 1 : 12. The complex therefore contains about one protein molecule of 35,000 molecular weight for every ten single-stranded DNA nucleotides. Because ten nucleotides can span a distance of not more than 70 Å, whereas 32-protein may be as much as 120 Å long, adjacent molecules of 32-protein could overlap in the complex. Consistent with this expectation, it was shown previously that in crude extracts 32-protein binds cooperatively to single-stranded DNA-cellulose¹⁹.

To determine whether the purified 32-protein also binds cooperatively to DNA, two different concentrations of 32-protein (containing the same amount of tritiated 32-protein) were mixed with a constant amount (large excess) of fd DNA at an elevated salt concentration where the complex is only marginally stable. The results of sucrose gradient sedimentation analyses are shown in Fig. 1. It is clear that a 14-fold increase in 32-protein concentration dramatically increases its DNA affinity. This result requires that 32-protein molecules interact with each other in the complex (see caption to Fig. 1), and suggests a model in which there are two types of binding sites for 32-protein on single-stranded DNA: ten nucleotides adjacent to a previously bound molecule of 32-protein ("contiguous" site) and ten not adjacent to any previously bound molecule ("isolated site"). If this model is correct, the affinity of 32-protein for a contiguous site must be at least eighty times greater than its affinity for an isolated site, for strong cooperative binding is observed even in conditions where the number of isolated

sites available exceeds the number of contiguous sites by at least this factor (Fig. 1).

The highly cooperative nature of the DNA affinity of 32-protein should cause it to bind to DNA in long clusters even in conditions of large DNA excess. Direct evidence for such clustered binding is obtained when labelled 32-protein is mixed with a large excess of fd DNA and sedimented through sucrose gradients at low salt concentrations. In this experiment, a larger portion of the 32-protein sediments ahead of the main DNA peak, being tightly bound to a small fraction of the DNA molecules. The mean size of a 32-protein cluster in these conditions must therefore be an appreciable fraction of the length of an fd DNA molecule (6,600 nucleotides)²³. Clustered binding to poly dA can also be detected by this method, indicating that the cooperativity observed is the result of direct stabilizing interactions between adjacent 32-protein monomers. This view is also supported by our finding (unpublished) that 32-protein self-aggregates in the absence of DNA at a concentration of 0.5 mg/ml. or higher.

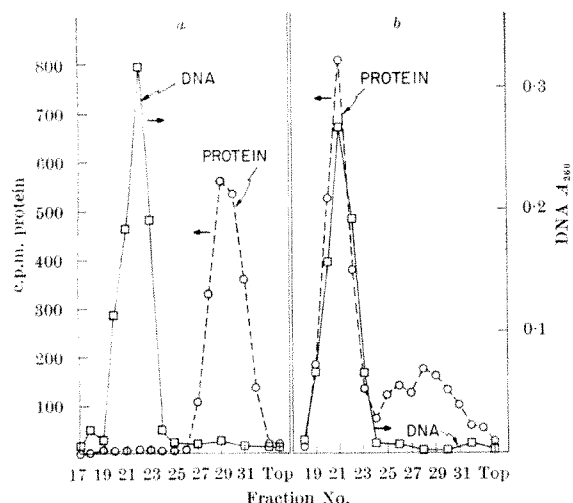


Fig. 1. Cooperative binding of 32-protein to single-stranded DNA. Purified fd DNA (10 μ g) was mixed with about 0.5 μ g (a) or 7 μ g (b) of 3 H-labelled 32-protein in 0.2 ml. of 0.02 M Tris-HCl (pH 8.1)—0.5 mM Na_2EDTA —0.30 M NaCl—100 μ g/ml. bovine serum albumin (BSA)—10 per cent glycerol—1 mM β -mercaptoethanol at 4° C. After 20 min. the mixture was layered at 4° C onto a 5 ml., 5–30 per cent sucrose gradient prepared in the same buffer. Following centrifugation for 2 h at 46,000 r.p.m. in the Spinco SW50 rotor, 0.15 ml. fractions were collected and monitored for radioactivity by standard techniques. Recoveries of 3 H-protein added averaged about 75 per cent. Concentrations of 32-protein ($A_{260} = 1.1$ mg/ml.) and fd DNA ($A_{260} = 23.8$ mg/ml. in 0.15 M NaCl—0.015 M sodium citrate, pH 7)²⁴ were determined by absorbance measurements. Note that, for ordinary binding, the protein distribution would have been identical in the above two experiments, for (free protein)/(bound protein) = K /(free DNA sites), and the concentration of free DNA sites was held essentially constant.

Although 32-protein binds very tightly to all single-stranded DNAs tested, including the synthetic polynucleotide poly dA, no binding of the purified protein to double-stranded DNAs or to R17 RNA could be detected by sucrose gradient sedimentation at 4° C.

Denaturation of DNA with 32-Protein

Histones and polyamines bind to the double-helical form of DNA more tightly than to single strands and thereby raise the temperature required for DNA denaturation^{25,26}. Conversely, the strong selective affinity of 32-protein for single-stranded DNA should lower the thermal denaturation temperature of double-stranded DNA. A precedent for such an effect is the destabilization of DNA observed in the presence of pancreatic ribonuclease which likewise preferentially binds to DNA single strands²⁷.

Because single-stranded DNA is fully hyperchromic when complexed with 32-protein, any denaturation of

double-stranded DNA which occurs in its presence should be accompanied by the large hyperchromic change that is characteristic of this helix-coil transition. By this criterion, double-stranded T4 DNA is not denatured in the presence of excess 32-protein in a variety of ionic conditions at temperatures up to 37° C. By contrast, poly dAT, which normally has a T_m about 16° C lower than T4 DNA (65° C as against 81° C in 0.01 M KCl—0.01 M MgSO_4), is readily denatured by 32-protein even at 25° C. Typical kinetics for poly dAT denaturation by 32-protein are shown in Fig. 2. In the presence of 0.01 M Mg^{2+} , half-denaturation of poly dAT by 32-protein is attained in about 20 min in the conditions used; this denaturation is reversible, for the absorbance at 260 nm can be restored to its original value either by addition of NaCl to 0.5 M at 25° C (to dissociate 32-protein), or by direct cooling to 4° C. The initial rate of denaturation at 25° C is reduced with increasing $[\text{Mg}^{2+}]$, decreasing at least 15-fold when $[\text{Mg}^{2+}]$ is increased from 0.01 M to 0.04 M, and increasing about three-fold when all Mg^{2+} is removed.

To denature poly dAT at 25° C, ΔG for the coil \rightarrow complex reaction (single-stranded DNA coil + 32-protein \rightarrow DNA-protein complex) must be sufficiently negative to make $\Delta G_{\text{helix} \rightarrow \text{coil}} + \Delta G_{\text{coil} \rightarrow \text{complex}} < 0$ at that temperature. The $\Delta G_{\text{helix} \rightarrow \text{coil}}$ should be about +1.0 kcalories/mole base-pairs for poly dAT in 0.01 M Mg^{2+} at 25° C ($T_m = 65^\circ \text{C}$) (ref. 28). Consequently, to obtain denaturation, $\Delta G_{\text{coil} \rightarrow \text{complex}}$ will need to be < -5 kcalories/mole of binding sites (ten single-stranded DNA nucleotides)

because $K_{\text{dissociation}} = \exp \left(-\frac{\Delta G/RT}{\frac{\text{free protein}}{\text{bound protein}} \cdot \frac{\text{free sites}}{\text{bound sites}}} \right)$

half-denaturation of poly dAT in 0.01 M Mg^{2+} at 25° C with 170 μ g/ml. of 32-protein (Fig. 2) will require an effective dissociation constant for 32-protein of $< 1.1 \times 10^{-9}$ M. This is in agreement with direct measurements in sucrose gradients in similar conditions, which yield a dissociation constant (averaged for cooperativity) of $< 10^{-9}$ M for the 32-protein complex with fd DNA.

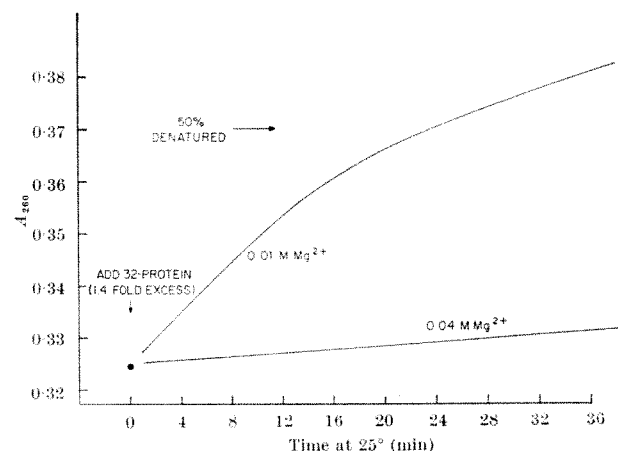


Fig. 2. Poly dAT denaturation catalysed by 32-protein at 25° C. In addition to the concentration of MgSO_4 indicated, each sample contained 10 μ g/ml. of poly dAT (from A. Kornberg, $s_{20,w} = 29$ in 0.1 M NaOH—0.9 M NaCl), 170 μ g/ml. 32-protein, 0.01 M KCl, 2 mM Tris-HCl (pH 8.1), 1 mM β -mercaptoethanol, 0.1 mM Na_2EDTA , and 2 per cent glycerol. Samples were placed in cuvettes in the thermostated compartment of a Gilford spectrophotometer, so that the absorbance could be monitored automatically. At time zero, concentrated 32-protein was added to start the reaction. Similar results have been obtained at a KCl concentration of 0.12 M.

Renaturation of DNA with 32-Protein

As already noted, the renaturation of purified DNA in physiological conditions *in vitro* is an extremely slow process, because of the intrastrand folding in denatured DNA. Our results imply, however, that in the T4 system single-stranded DNA does not exist as such *in vivo*, but is instead always present as a tight complex with 32-

protein. We find that fd DNA saturated with 32-protein sediments only about 1.3 times faster than the free DNA, although its mass is thirteen times greater. This means that the frictional coefficient of fd DNA increases about six-fold in the complex. Because frictional coefficients only double when single-stranded DNA is unfolded in alkali²⁹, complexed DNA must be held in a highly expanded conformation by 32-protein. (This expansion can be seen directly by electron microscopy; personal communication of H. Delius.) DNA in such a conformation might be expected to renature much more rapidly than free denatured DNA at low temperatures.

The rate of renaturation of DNA covered with 32-protein was measured by an absorbance assay similar to

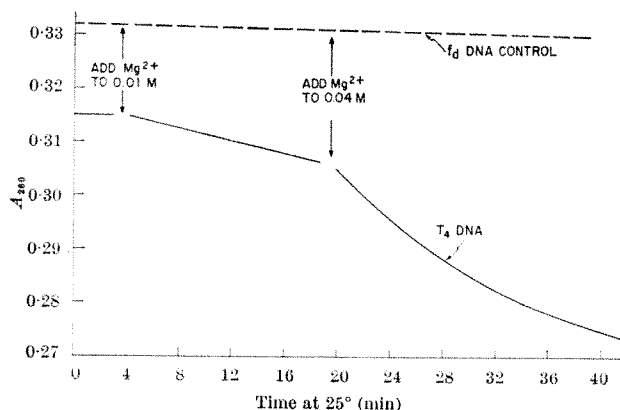


Fig. 3. DNA renaturation catalysed by 32-protein at 25° C. Single-stranded T4 DNA was prepared by alkaline denaturation and dialysis into low salt buffer as described by Studier¹³, except that the DNA was sheared in alkali to molecular weight 5.4×10^6 before the dialysis. The fd DNA serves as a control, for it is not self-complementary and therefore cannot renature. At zero time, 10 $\mu\text{g}/\text{ml}$ of each DNA was mixed in separate cuvettes with 170 $\mu\text{g}/\text{ml}$ of 32-protein in the buffer used in Fig. 2. At the times indicated, MgSO_4 was added from a 1 M stock to both fd and T4 DNA reactions. A decrease in absorbance of about 0.072 units is expected for full renaturation of the T4 DNA.

that used to monitor denaturation in Fig. 2. In this case, a decrease in the absorbance of single-stranded DNA is expected proportional to the amount of reformed double-helix. Typical results obtained for single-stranded T4 DNA in the presence of excess 32-protein at 25° C are presented in Fig. 3. It can be seen that a rapid decrease in absorbance is observed in 0.04 M Mg^{2+} , representing a more than 1,000-fold acceleration of the renaturation rate without 32-protein. The dependence of this reaction on $[\text{Mg}^{2+}]$ is the reverse of that found for denaturation in Fig. 2: the renaturation rate drops about four-fold in 0.01 M Mg^{2+} , whereas no renaturation is detected without Mg^{2+} .

A more sensitive measure of the course of renaturation is obtained from CsCl gradients, where renatured DNA has a lower buoyant density than single strands¹⁴. This assay

Table 1. RENATURATION RATES FOR T₄ DNA SINGLE STRANDS OF MOLECULAR WEIGHT 5.4×10^6

32-Protein	DNA concentration ($\mu\text{g}/\text{ml}$)	Ionic composition	Temperature	(K_2 l. mole ⁻¹ s ⁻¹)
—	15	1.0 M NaCl	68°	270
—	76	0.04 M MgSO_4	37°	< 0.2
+	15	0.01 M KCl	37°	300
+	72	0.011 M MgSO_4	37°	22
		0.12 M KCl		

For the 32-protein catalysed reaction: (1) renaturation rates decrease with storage of the protein, so that the maximum rates are probably greater than those listed; (2) between 0.01 M and 0.04 M Mg^{2+} , the rate at 37° C is roughly proportional to $[\text{Mg}^{2+}]$; it falls drastically at lower Mg^{2+} levels; (3) renaturation rates are reduced as $[\text{KCl}]$ is increased, being severely affected above 0.15 M; and (4) addition of spermidine (0.001 M) is without significant effect. The rates shown were measured by CsCl banding after incubation at pH 7.6 (the catalysed reaction has a broad optimum between pH 7 and pH 8). Enough 32-protein was used fully to saturate the DNA.

can be used for kinetic analyses, for the addition of concentrated CsCl dissociates 32-protein from the DNA and prevents further renaturation. By this technique, it has been found that the rate of renaturation of T4 DNA in the presence of excess 32-protein is proportional to the square of the DNA concentration, showing that, as in the uncatalysed reaction, the rate measured is that for the nucleation of complementary pairings. Some second order rate constants for the renaturation of T4 DNA single strands of molecular weight 5.4×10^6 are listed in Table 1. Note that the rate of renaturation catalysed by 32-protein at 37° C can exceed the uncatalysed rate observed in standard conditions (68° C in 1 M NaCl).

If 32-protein accelerates DNA renaturation by imparting favourable conformation to the single strands, the bases of which would otherwise be inaccessible on highly folded chains, the dependence of renaturation rate on the ratio of 32-protein to DNA should be unusual. Below the saturating protein : DNA ratio of 12 : 1, the rate of renaturation should be drastically lowered, initial rates being proportional to at least the square of the amount of 32-protein added. Results of renaturation assays performed at sub-saturating 32-protein levels are shown in Fig. 4, where it is seen that a four-fold drop in the concentration of 32-protein decreases the rate of renaturation of T4 DNA at least twenty-five-fold, as expected. A second expectation is that above a protein : DNA ratio of 12 : 1, additional 32-protein should not further increase renaturation rates. This prediction has also been confirmed (experiment not shown).

On the basis of these results, we conclude that 32-protein accelerates renaturation in physiological conditions by forcing DNA single strands into an unfolded conformation which leaves their bases available for pairing during chance collisions between complementary strands. It seems likely that the bound 32-protein is rapidly displaced from the rewinding single strands as the double-helix forms (see Fig. 3).

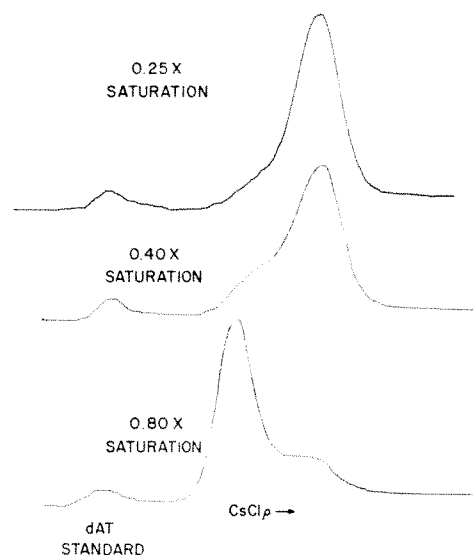


Fig. 4. T4 DNA renaturation as a function of 32-protein : DNA ratio. Renaturation was carried out with T4 DNA single strands (Fig. 3) at 72 $\mu\text{g}/\text{ml}$, and the quantity of 32-protein added was varied as indicated. Incubation was at 37° C in buffer containing 0.12 M KCl and 0.011 M MgCl_2 . After 30 min, the reaction was quenched by addition of concentrated CsCl (to $\rho = 1.700$) plus 50 μg of sodium dodecyl sulphate. The band patterns shown are tracings of photographs taken after centrifugation for 20 h at 44,770 r.p.m. in the Spinco model E analytical ultracentrifuge. The renatured band has shifted to a buoyant density representing molecules which are about two-thirds double-helical, as expected from the pairing of strands randomly cut from a longer sequence³⁰. As controls, no density shift was observed when fd DNA was processed in an identical manner, whereas fragments of single-stranded T7 DNA renatured rapidly in the presence of 32-protein.

Function of 32-Protein in Genetic Recombination

The denaturation of poly dAT by 32-protein at 25° C indicates that within the cell, the cooperative action of 32-protein should generate fluctuating regions of local denaturation in T4 DNA. The requirement for 32-protein in early steps of genetic recombination can be explained by this ability to open up local regions of native DNA, while simultaneously facilitating helix formation between matching, complexed single strands. Both of these functions are probably necessary for efficient testing of complementary pairings between double-stranded DNA molecules. In addition, experiments with infected cells have suggested that the formation of single-strand breaks ("nicks") in double-stranded intracellular T4 DNA is a prerequisite for the initiation of strand exchanges³¹. Order-of-magnitude calculations, based on the treatment of DNA renaturation formulated by Wetmur and Davidson³⁰, suggest that random collisions between specifically nicked, double-stranded T4 DNA molecules (see ref. 5) might be efficient enough in the presence of 32-protein to account for the high recombination rates observed in the T4 system (unpublished results). This, however, merely indicates that the mechanism of genetic recombination may be relatively simple, for the properties of 32-protein are also compatible with most other types of models suggested for the recombination process.

Function of 32-Protein in DNA Replication

The genetic results which indicate a structural role for 32-protein in DNA replication suggest that local unwinding by 32-protein might be required in the replication fork in order for productive replication to proceed. In agreement with this role, *in vitro* experiments have revealed that T4 DNA polymerase³² uses single-stranded DNA templates much more rapidly in the presence of 32-protein than in its absence (unpublished results of Huberman, Kornberg and B. M. A.). This stimulation is probably the result of favourable template alignment by 32-protein. Because stimulation is not observed in similar experiments with *E. coli* DNA polymerase, a direct interaction of T4 polymerase with 32-protein may also be involved.

In a normal T4 bacteriophage infection, the number of replication forks present per cell increases linearly until 30 min after infection (25° C)³³. Because the rate of polymerization observed at each fork is constant throughout this period³³, this rate must be independent of the level of 32-protein, inasmuch as this increases continuously during infection. Yet the gene dosage experiments, which reveal a direct proportionality between the quantity of phage progeny produced and the quantity of 32-protein present, seem to demand that the overall rate of DNA synthesis be proportional to the amount of 32-protein made²¹. To account for these facts, we propose that a functioning replication fork has a unique tertiary structure that contains a fixed number of 32-protein molecules. (About sixty new replication forks are eventually generated in a normal T4-infected cell, so that each fork could incorporate no more than 170 molecules of 32-protein.) In our view, the amount of 32-protein determines the quantity of DNA made, for a new replication fork can be formed only as fast as a threshold level of free 32-protein becomes available.

If each cycle of DNA replication begins at a special point on the T4 genome³⁴, new replication forks must be generated only at a unique nucleotide sequence. Both during this process and as the replication fork travels, 32-protein may interact with other proteins in addition to T4 DNA polymerase. Likely candidates for such proteins include the products of T4 genes 41, 44, 45, 59 and 62, all of which have as yet unidentified functions essential for T4 DNA replication¹⁷. Further studies involving 32-protein may therefore provide a fresh insight concerning the unknown mechanism by which DNA is replicated in biological systems.

Preparation of Homogeneous 32-Protein

An *E. coli* culture, grown to 5×10^8 cells per ml. at 32° C in M-9 minimal media containing 0.3 per cent casein hydrolysate plus 1 per cent glucose, was infected twice at 10 min intervals with a total m.o.i. of ten T4e bacteriophages (lysozyme⁻). The cells were harvested and washed after 90 min of aeration at 32° C and stored at -20° C. Cells (50 g) were broken by sonication after resuspension in 200 ml. of 0.02 M Tris-HCl (pH 8.1)—0.01 M MgCl₂—2 mM CaCl₂—1 mM β -mercaptoethanol—1 mM Na₃EDTA containing 20 μ g/ml. pancreatic deoxyribonuclease I (Worthington). After incubation for 90 min at 10° C, the extract was centrifuged at low speed to remove cell debris and then clarified at 30,000 r.p.m. for 3 h in the Spinco 30 rotor. The supernatant was dialysed for 24 h against several changes of 0.02 M Tris-HCl (pH 8.1)—0.05 M NaCl—5 mM Na₃EDTA—1 mM β -mercaptoethanol (buffer A) to remove the divalent cations necessary for the activity of deoxyribonuclease I. After centrifugation to remove a light precipitate, the dialysed extract was made 10 per cent in glycerol and forced at 100 ml/h through a column containing 20 ml. packed volume of denatured calf thymus DNA-cellulose (approximately 1 mg of DNA per ml.). The DNA-cellulose (7 cm \times 3 cm²) had been equilibrated with a buffer consisting of 10 per cent glycerol in buffer A, and this basic buffer was used for an 80 ml. rinse and for elutions in which increasing concentrations of NaCl were added. The column was eluted at 20 ml/h, in 40 ml. steps of 0.15, 0.40, 0.60 and 2.0 M NaCl. The peak 2.0 M NaCl eluting fraction contained 32-protein as its principal component. This fraction (8 ml.) was dialysed against 0.02 M Tris-HCl (pH 8.1)—10 per cent glycerol—1 mM Na₃EDTA—1 mM β -mercaptoethanol (buffer B) and applied to a 7 cm \times 0.8 cm² column of DEAE-cellulose (Whatman DE32). The column was washed with 5 ml. of buffer B and then eluted with a 30 ml. linear gradient of 0.0–0.5 M NaCl in this buffer. Fractions of 1.3 ml. were collected every 20 min. The 32-protein (A_{280}/A_{260} absorbance ratio of 1.7) appeared in three adjacent fractions with a mean NaCl concentration of 0.20 M. These fractions were either used directly for the studies to be described, or concentrated further by vacuum dialysis against buffer B containing 0.05 M KCl. Approximately 8 mg of electrophoretically homogeneous 32-protein is obtained by this procedure. As determined by the subsequent recovery of purified ³H-labelled 32-protein added to crude extracts, this represents about a 65 per cent yield. An identical procedure was used on a smaller scale for preparation of ³H-labelled 32-protein, except that the cells were grown at 25° C and labelled with 500 μ Ci of ³H-leucine after 35 min of infection. Unless otherwise stated, all operations were carried out at 4° C; at this temperature, concentrated solutions of 32-protein (> 500 μ g/ml.) may be kept for several weeks. At -80° C, 32-protein has been stored for up to 8 months without a noticeable change in its DNA affinity.

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Three Variable-Gene Pools common to IgM, IgG and IgA Immunoglobulins

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Comparison of the NH₂-terminal region of several α , γ and μ chains indicates that four variable-sequence subgroups are common to heavy chains. The existence of three independent variable-gene pools common to the three major immunoglobulin classes is confirmed.

IMMUNOGLOBULINS can be divided into three principal classes according to their different antigenic and functional properties. The class differences reside in the heavy chains, whereas the light chains, which are chemically and immunologically defined as κ or λ chains, are shared by all three immunoglobulin classes^{1,2}. The IgA immunoglobulin class is characterized by the α chain, IgG by the γ chain and IgM by the μ chain. In spite of these structural and functional differences certain common features exist for the three chief classes. A common evolution for all immunoglobulins is evident from comparison of the chemical structure of heavy and light chains from different species^{2,3}. Furthermore, there is a general cooperative and sequential relationship in the immune response of all three immunoglobulin classes. Finally, serological cross-reactions between IgM and IgG have been observed by a number of workers. In fact, the earliest evidence for a heavy chain gene was the finding by Todd⁴ of a common allotypic marker in rabbit IgG and IgM. Thus the question arises where in the primary structure of the α , γ and μ chains the differences and similarities of the three principal immunoglobulin classes are localized. Earlier⁵ we made the first comparison of an extended sequence of a μ chain (Ou) with a γ 1 chain (Daw)⁶ which showed that in the first 105 NH₂-terminal residues of these two human heavy chains of different classes the homology in sequence was almost 75 per cent. Subsequently, we undertook sequence analysis of several human IgM Waldenström macroglobulins and IgA myeloma proteins to ascertain if this high degree of homology of two different heavy chain classes was merely accidental. We now report additional heavy chain sequences from the NH₂-terminus of four IgM macroglobulins and one IgA myeloma globulin. Comparison of these data with the NH₂-terminal sequences of heavy chains from other laboratories has revealed homologies of different degrees which suggest the existence of two new variable heavy chain subgroups, V_{HIII} and V_{HIV} in addition to the V_{HI} and V_{HII} subgroups already proposed^{7,8}.

Determination of Amino-acid Sequence

Because most of the heavy chains in immunoglobulins have a blocked NH₂-terminal residue which is the cyclized form of glutamine (pyrrolidone carboxylic acid or PCA)^{9,10}, a specific method to isolate blocked NH₂-terminal peptides from the whole molecule can be used¹¹. IgM protein (1–2 μ moles) was digested with subtilisin for 2 h at 37° C and pH 8. The soluble digest was then applied to a 'Dowex 50 \times 2' column previously washed with 1 M HCl and distilled water until neutral. The first peak eluted contained glycopeptides and the second peak a tetrapeptide which was ninhydrin-negative on paper. The amino-acid composition of the tetrapeptide from proteins Ou, Di and Re varied in only one residue. In all three cases a 10 min and 120 min incubation with carboxypeptidase A released two residues in different yields. By this method the amino-acids at positions three and four were established. Because the first residue could be assumed to be PCA, the residue in position two could be deduced. In another experiment, we determined the partial sequence of the nineteen NH₂-terminal residues of protein Di by study of tryptic peptides from the NH₂-terminal fragment obtained by CNBr cleavage.

Attempts to isolate a blocked peptide failed with the IgM proteins Wo (personal communication from A. van Dalen) and Na and with the IgA protein Ha. But we found a free NH₂ terminus on these heavy chains by means of the dansylation technique¹². The NH₂-terminal residue in the unblocked heavy chains of proteins Wo, Na and Ha was glutamic acid. These results confirm earlier data from this laboratory which showed that in about half of the μ chains studied the NH₂ terminus was not blocked¹⁰. The μ chains of IgM Wo and Na and the α chain of IgA Ha were subjected to the automatic Edman degradation method¹³ using the Beckman sequencer model 890. Identification of the degraded PTH-amino-acid was done by thin-layer chromatography¹⁴ and by gas chromatography using a combination of three different columns¹⁵. In one

case (Wo) up to nineteen steps were identified unambiguously.

Four Subgroups of Heavy Chain Variable Sequences

When previous sequence data on several γ chain myeloma proteins are compared with our own results on μ and α chains, four groups of homologous sequences can be recognized by comparison of as few as the first twenty residues (Fig. 1). In the first group (V_{HI}) one new μ chain (Di) from our laboratory is aligned with three γ chains reported by others (Eu¹⁶, Zuc¹⁷ and Ste¹⁸). Approximately 75 per cent of the residues are identical. In the second group (V_{HII}) three myeloma $\gamma 1$ chains (Daw⁶, Cor¹⁹ and He⁸) are compared with the Ou μ chain from our labora-

their first twenty residues. On the other hand, the distance between the three subgroups, as measured by characteristic differences in sequence, is from seven to ten amino-acid residues in the first twenty.

The fourth subgroup proposed (V_{HIV}) is rather miscellaneous, for it comprises the predominant sequence of two γ chain allotypes from the rabbit²¹ and a number of short μ chain sequences in man²² including the μ chain Re from our laboratory. This heterogeneous subgroup, which may have to be subdivided in the future, has a serine residue at position 2 as its most characteristic feature.

All four subgroups show marked differences one from another; yet there is a basic sequence underlying all four that is most evident in V_{HI} , V_{HII} and V_{HIII} . Because of

	5	10	15	20
V_{HI}				
Eu $\gamma 1$	PCA-Val-Gln-Leu-Val-Gln-Ser-Gly-Ala-Glu-Val-Lys-Lys-Pro-Gly-Ser-Ser-Val-Lys-Val			
Di μ	PCA-Val-Gln-Leu(Thr, Glx, Ser, Gly, Ala, Gly, Leu)Lys-Lys(Pro, Gly, Glx, Pro —)Lys			
Zuc $\gamma 3$	PCA-Val-Gln-Val-Val-Glu-Ser-Gly-Ala- <u>Asp</u> -Leu-Val-Lys-Pro-Gly-Gly			
Ste $\gamma 1$	PCA-Val- <u>His</u> -Leu-Val-Glu-Ser- <u>Ser</u> -Ala-Glu-Val-Lys-Lys-Pro-Gly- <u>Ala</u> -Ser- <u>Met</u> -Lys-Val			
V_{HII}				
Daw $\gamma 1$	PCA-Val-Thr-Leu-Arg-Glu-Ser-Gly-Pro-Ala-Leu-Val-Arg-Pro-Thr-Gln-Thr-Leu-Thr-Leu			
Ou μ	PCA-Val-Thr-Leu- <u>Thr</u> -Glu-Ser-Gly-Pro-Ala-Leu-Val- <u>Lys</u> -Pro- <u>Lys</u> -Gln- <u>Pro</u> -Leu-Thr-Leu			
Cor $\gamma 1$	PCA-Val-Thr-Leu-Arg-Glu-Ser-Gly-Pro-Ala-Leu-Val- <u>Lys</u> -Pro-Thr-Gln-Thr-Leu-Thr-Leu			
He $\gamma 1$	PCA-Val-Thr-Leu- <u>Lys</u> -Glu- <u>Asn</u> -Gly-Pro- <u>Thr</u> -Leu-Val- <u>Lys</u> -Pro-Thr-Glu-Thr-Leu-Thr-Leu			
V_{HIII}				
Vin $\gamma 4$	Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Gly-Leu-Ile-Gln-Pro-Gly-Gly-Ser-Leu-Arg-Leu			
Wo μ	Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Gly-Leu- <u>Val</u> -Gln-Pro-Gly-Gly-Ser-Leu-Arg-Leu			
Na μ	Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly- <u>Ala</u> -Leu			
Ha $\alpha 1$	Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Gly-Leu- <u>Val</u> -Gly-Pro-Gly			
V_{HIV}				
rabbit Aa1	PCA-Ser-Val-Glu-Glu-Ser-Gly-Gly-Arg-Leu-Val-Thr-Pro-Thr-Pro-Gly-Leu-Thr-Leu-Thr			
rabbit Aa3	PCA-Ser- <u>Leu</u> -Glu-Glu-Ser-Gly-Gly- <u>Asp</u> -Leu-Val- <u>Lys</u> -Pro- <u>Gly</u> - <u>Ala</u> -Ser-Leu-Thr-Leu-Thr			
Dos μ	PCA-Ser-Val- <u>Ala</u> - <u>Asx</u>			
Bu, Dau μ	PCA-Ser-Val- <u>Leu</u> - <u>Asx</u>			
Bal μ	PCA-Ser-Val- <u>Ala</u>			
Re μ	PCA-Ser- <u>Ala</u> - <u>Leu</u>			

Fig. 1. Comparison of the four variable subgroups from the γ , α and μ chains of human myeloma globulins and Waldenström macroglobulins and from normal rabbit γ globulin allotypes. Sequences of proteins from other laboratories are taken as follows: Daw⁶, Cor¹⁹, Ste¹⁸, He⁸, Eu¹⁶, Zuc¹⁷, Vin²⁰, rabbit Aa1 Aa3 allotypes²¹, Dos, Bu, Dau and Bal²²; underlined residues differ from the sequence in the first line of each subgroup. Only the principal residues are given for the sequence of the rabbit heavy chains.

tory⁸. The identity in sequence of the first twenty amino-acid residues of this group varies between 66 per cent and 75 per cent. The same correspondence in sequence continues throughout the first 100 residues of these four members of subgroup V_{HII} , but all four have only about a 30 per cent identity in sequence with the Eu $\gamma 1$ heavy chain throughout the whole variable region which comprises about 115 residues. This shows that the amino-acid sequence characteristic of the subgroup can be identified from the sequence of the first twenty residues of heavy chains, just as it can for light chains.

The third subgroup that we have identified is designated V_{HIII} ; it includes all three classes of heavy chains (Vin $\gamma 4$, Wo μ , Na μ , Ha α) and is characterized by the presence of a free NH_2 -terminal group of glutamic acid. Evidence of the close identity between the Vin $\gamma 4$ chain²⁰ and the Wo μ chain is the fact that there is only one difference in

this apparent division of the variable sequences of μ , γ and α chains into four subgroups, the two new variable heavy chain subgroups, V_{HIII} and V_{HIV} , may be added to the two already proposed^{7,8}.

As stated elsewhere^{8,23}, the class character of heavy chains is not expressed in the variable region (V_H) but only in the constant region (C_H). This is by contrast with the situation in light chains, where both the variable and the constant regions carry the signature of the light chain type, κ or λ . Formulae for immunoglobulins have recently been proposed²⁴ in which the variable regions of IgG, IgA and IgM are provisionally designated V_γ , V_α and V_μ . The results reported here, however, show that the variable regions of γ , α and μ heavy chains do not fall into groups that can be classified by the nature of the C region of the heavy chain (C_γ , C_α and C_μ) but rather fall into subgroups that are independent of the C regions.

Because the V_H regions of μ chains cannot be put into a group apart from the V_H regions of γ or α chains, we have used the symbols V_{HI} , V_{HII} , V_{HIII} and V_{HIV} to designate the subgroups of the variable regions of all three classes of heavy chains.

Variable Regions of Light and Heavy Chains on the Same Molecule

Recently⁷ we compared the variable regions of heavy and light chains from the same immunoglobulin molecule. Our new sequences from the μ chains of IgM Di, Na, Re and Wo and the α chain of IgA Ha permit us to extend the comparison of light and heavy chains in the same molecule. The NH_2 -terminal thirty residues of the light chain of the IgM Di were determined with the automatic Edman degradation method¹³ and will be reported elsewhere. This sequence resembles the $V_{\lambda III}$ subgroup. The complete sequence of the Ha light chain has already been reported from this laboratory²⁵; it belongs to the $V_{\lambda I}$ subgroup. The light chain of IgM Re was identified by us as being of the $V_{\lambda II}$ subgroup. Serologically and chemically the light chains of IgM Na¹⁰ and IgM Wo¹² were defined as κ types. Fig. 2 represents schematically the combinations of variable light and heavy chain types found in eight different human immunoglobulin molecules of all three principal classes IgA, IgG and IgM. Apparently, any variable light chain subgroup regardless of the immunoglobulin class. This finding is evidence that the evolution of heavy and light chains proceeded independently. Furthermore, this independent evolution of both chains must have occurred in separate variable and constant gene lines which represent the different variable subgroups and constant immunoglobulin class types.

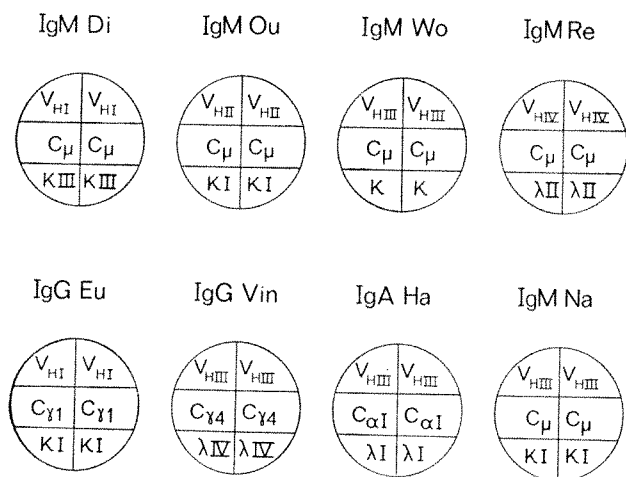


Fig. 2. Schematic representation of different immunoglobulin molecules. The model for the IgG protein Eu is based on the results of Edelman *et al.*¹⁴ and for the IgG protein Vin on the work of Pink and Milstein²⁰. The circles symbolize the tetrachain polypeptide structure for each molecule. In the upper part of each circle the variable heavy chain subgroup is given; in the lower part the light chain type or subgroup.

Variable-gene Pools common to IgM, IgG and IgA

For the variable subgroups of light and heavy chains of the three principal immunoglobulin classes, we propose the existence of three different variable-gene pools. The first, the heavy chain variable-gene pool, is common to all α , γ and μ chains and comprises the four subgroups V_{HI} , V_{HII} , V_{HIII} and V_{HIV} . The second variable-gene pool codes for the κ chains only (κI , κII and κIII), and the third pool for the λ chains (λI , λII , λIII , λIV and λV).

From sequence comparison of subgroups from these three pools, we have observed a weak homology between the V_{HIV} subgroup and λI and λII subgroups and also between the V_{HIII} and the κIII subgroups. This weak homology indicates a very early separation in the evolution of variable genes for light and heavy chains.

The existence of a common variable-gene pool for μ , α and γ chain biosynthesis would obviously reduce the number of genes needed for the immunoglobulins and thus would accord with a principle of economy in nature. The proposed common variable-gene pool for μ and γ chains could also explain the biological and serological relations between IgM and IgG; for example, Todd's finding⁴ of common allotypic genetic markers in rabbit IgM and IgG and Feinstein's finding of the same marker in rabbit IgA²⁶. It has been shown that these allotypes are on the Fd heavy chain regions^{26,27} and amino-acid sequence differences have been reported²¹ for the variable regions of rabbit allotypes Aal and Aa3. Unlike the individually unique amino-acid sequences reported here for the variable regions of human immunoglobulins, however, the rabbit allotype sequences are mixed, for example, only the predominant residues are shown in Fig. 1. Some workers²⁸ have thus questioned whether these differences are genuinely associated with serologically determined allotypic markers. Indeed, in our proposed classification both rabbit allotypes Aal and Aa3 fall into the same variable subgroup, V_{HIV} . The amino-acid sequences reported here for the variable regions of individual human myeloma globulins and macroglobulins confirm and extend the division of the V-region of human heavy chains into subgroups shared by γ , α and μ chains and give further support to the hypothesis that there are three variable gene pools common to IgG, IgA and IgM immunoglobulins.

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Nucleotide Sequences of Sections of 16S Ribosomal RNA

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Three large sections of 16S RNA, obtained by partial enzymatic hydrolysis, and together representing nearly one-fifth of the molecule, have been analysed. Knowledge of their primary structure allows features of the secondary structure to be envisaged.

SINCE the introduction of techniques for the rapid fractionation of radioactive oligonucleotides¹, it has been possible to begin studying the primary structures of very large RNA molecules. In this laboratory we have used

these methods to examine the large ribosomal RNAs of *Escherichia coli*, with a view to determining the nucleotide sequences of extensive areas of these molecules. We believe that such information about the primary structures will give some indications of the secondary structures of these molecules, and perhaps lead to some idea of the topography of ribosomal particles. Additionally, knowledge of the primary sequences would be essential in any attempts to describe the specificity of the interactions between the ribosomal proteins and the RNAs, during the assembly of ribosomes.

We have previously reported the nucleotide sequences of products arising from complete enzymatic digestions of the 16S ribosomal RNA^{2,3}. In this article we report the nucleotide sequences of three large sections of this molecule. These sequences encompass 284 nucleotides, 17–18 per cent of the total length of the 16S RNA. We suggest that these regions would be likely to possess extensive and characteristic secondary structures.

We used ³²P-labelled 16S RNA, which was prepared from *E. coli* (MRE 600) in the way previously described⁴. About 5 mCi of 16S RNA was obtained in each preparation from a culture grown in the presence of 60 mCi of ³²P-orthophosphate. The 16S RNA was subjected to partial hydrolysis with T₁ ribonuclease, and the resulting fragments were fractionated by electrophoresis through large slabs of 12 per cent polyacrylamide gel^{5,6}.

Autoradiograms of two such fractionations are shown in Fig. 1. A fairly reproducible range of products was obtained in the conditions of digestion used (see the legend to Fig. 1). In the first instance we examined many of the fragments resulting from this procedure by digesting them with T₁ and pancreatic ribonucleases, and fingerprinting the products. These oligonucleotides were then identified by further enzymatic digestions, with reference to the complete oligonucleotide maps of the 16S RNA which we had previously compiled. In this way we determined which oligonucleotides of known sequence were present in each of the fragments marked on Fig. 1. We also carried out quantitative studies of the amounts of the different oligonucleotides arising from each fragment. In general these products arose in molar quantities from the larger fragments, and the sizes of these fragments which could be calculated from the quantitative data corresponded with their relative positions after gel electrophoresis. On this basis, the larger fragments, including all those marked on Fig. 1, should have been reasonably pure, and suitable for further sequence analysis without additional fractionation. From these studies it also became apparent that many of the fragments were related to each other. This is discussed in the legend to Fig. 4.

We have determined the complete nucleotide sequences of fragment "4" and the overlapping fragment "200", together encompassing 174 nucleotides (section A), and

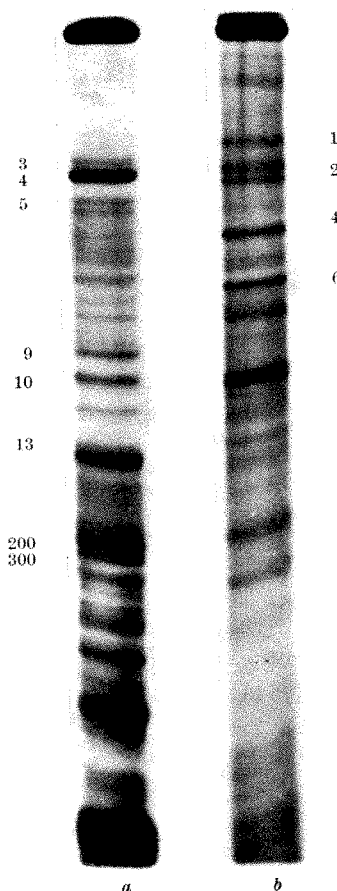


Fig. 1. The fractionation of large fragments of the 16S RNA by electrophoresis through slabs of polyacrylamide gel. The gels were prepared in the way described by Adams *et al.*⁶, using solutions containing 11.4 per cent acrylamide and 0.6 per cent bis-acrylamide. The dimensions of the gels were 40 × 15 × 0.3 cm, and the slots used for applying the RNA measured 2.5 × 1 × 0.1 cm. The following electrophoretic buffer system was used: 0.01 M Tris borate (pH 8.3)—0.0025 M EDTA. The 16S RNA was dissolved in 0.05 ml. of 0.01 M Tris-HCl (pH 7.4), to give a solution containing 10–15 mg/ml. of RNA. Hydrolysis with T₁ ribonuclease was carried out for 30 min at 0° C. In the examples shown above (different runs), the following enzyme-substrate ratios were used: (a) 1: 500; (b) 1: 1,000. After digestion the hydrolysate was mixed with an equal volume of a 20 per cent sucrose solution, saturated with urea, and containing a small amount of bromophenol blue marker dye. It was rapidly applied to the gel, and electrophoresis was started immediately. Electrophoresis was continued for 16 h at 4° C, with a current of 25 mA, and a potential difference of 10 V/cm. Autoradiography of the gel was carried out for 4 min. The bands were excised on the basis of the autoradiograms, and eluted electrophoretically⁷.

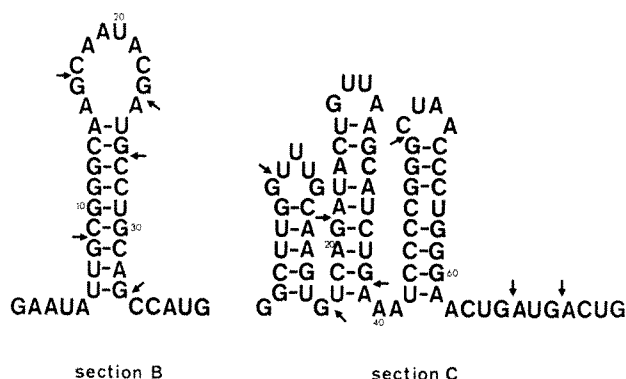


Fig. 2. The nucleotide sequences of section B (fragment "300") and section C (fragment "10"). The sequences shown here are each about five nucleotides shorter than the corresponding fragments marked on Fig. 1a, for in most of our partial digests these fragments were encountered without these additional nucleotides. The sites where hydrolysis occurred during partial digestions are indicated by arrows.

also the sequences of fragments "10" (section C, 72 nucleotides) and "300" (section B, 38 nucleotides). Sequence analysis of these fragments was carried out by subjecting them to further partial digestion with T_1 ribonuclease. The resulting products were fractionated by electrophoresis through slabs of polyacrylamide gel. We have used gels made up in two layers, of 15 per cent and 20 per cent polyacrylamide respectively. This system has been used to purify fragments containing 10–130 nucleotides. The products fractionated in this way were then further characterized by complete digestion with T_1 and pancreatic ribonucleases. The products arising in particular conditions of digestion could be obtained reproducibly, and most of the products which we used in the sequence analysis of these sections were obtained several times.

The sequences of these sections are set out in Figs 2 and 3, and we have also suggested possible secondary structures for each of them. These secondary structures are proposed on the basis of their sequences and their observed susceptibilities to hydrolysis during partial enzymatic digestion. Section B can be readily envisaged as a single hairpin loop, excised intact during partial digestion of the 16S RNA; section C can be thought of as an assembly of three such loops. We believe that most of section A possesses a more complicated secondary structure, with additional base-pairing of the connecting regions between the loops. This is supported by two sorts of observation: first, this fragment is always obtained in much greater amounts than other large fragments in the fairly harsh conditions of partial hydrolysis used in Fig. 1a, suggesting that it is especially resistant to enzymatic digestion because it contains a high proportion of secondary structure. On the basis of its nucleotide sequence, however, fragment "4" can only contain considerable secondary structure if an arrangement of the type shown in Fig. 4 is invoked. Second, the points at which we have observed hydrolysis to occur in conditions of mild partial enzymatic digestion are in the main situated in regions which are not base-paired in the structure proposed in Fig. 3. In all of the sections, most splitting which has been observed within the proposed helical regions during partial digestion is not within runs of several G-C pairs, but adjacent to A-U or G-U pairs. In the secondary structures proposed here, the amount of base-pairing and the overall proportion of G-C pairs are similar to the values gained from spectrophotometric studies of the secondary structure of the 16S RNA from reticulocytes⁷. In those parts of the sequences where no further base-pairing is likely to occur (1–117 of section A and 1–62 of section C) 65 per cent of the nucleotides participate in base-pairing. The mean amount of G-C pairs in the helical regions is 53 per cent, although considerable variation is apparent.

If the secondary structure proposed for fragment "4" is essentially correct, the simplest explanation of its occurrence among the products of partial digestion of the 16S RNA is that it arises from a region close to the middle of the RNA chain, and that the molecule is folded back on itself, with the folding point lying within fragment "4". Large fragments arising from other parts of the molecule during partial digestion would necessarily be single loops, or several loops connected by nonbase-paired sections. We have some direct information about the area within the 16S RNA chain from which fragment "4" is derived. We have isolated a fragment containing the 5'-terminal oligonucleotide from a partial digest carried out on the 30S ribosomal subunit. From its complete digestion products, and its mobility in polyacrylamide gel electrophoresis relative to products of known sequence, it is likely that this fragment contained at least 150 nucleotides.

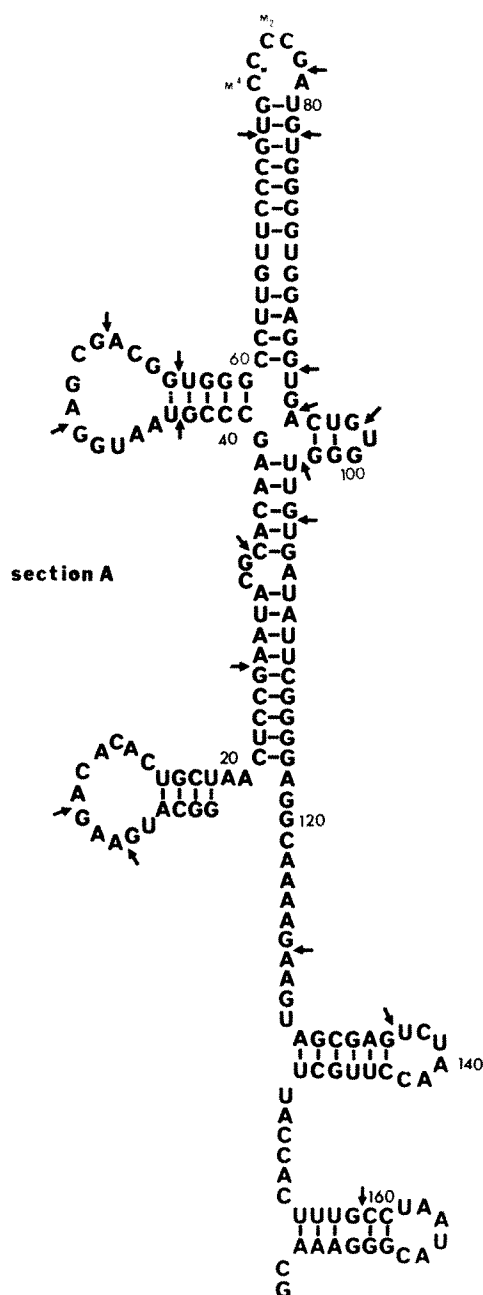


Fig. 3. The nucleotide sequence of section A. This section encompasses the overlapping fragments "4" (1–158) and "200" (127–174). Fragments "5" and "9" are subsections.

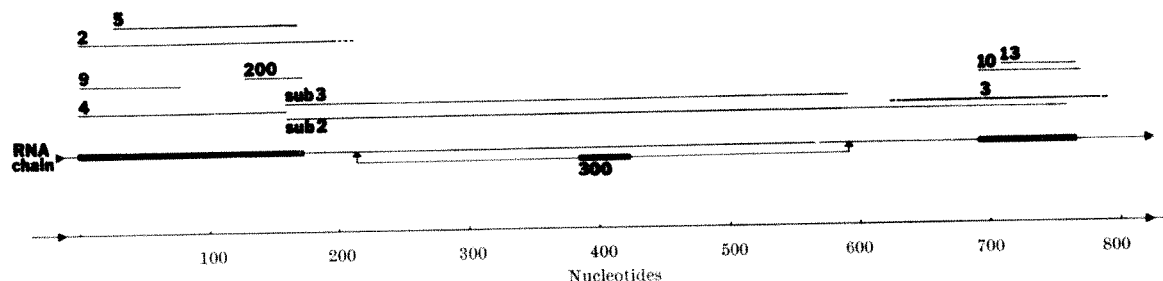


Fig. 4. The order of the nucleotide sequences within the 16S RNA. The location of section A is discussed in the text. Section B must occur within the fragment "sub-3", for it contains several unique sequences which are present in this fragment, but its precise location is not known. Section C probably represents the 3'-terminal segment of fragment "sub-2". The sequence GACUG is probably not present in "sub-2", but the sequence GAACUG is there. The 3'-terminus of "sub-2" must therefore lie between these points. Fragment "13" is a subsection of fragment "10", which is in turn a subsection of fragment "3". Fragment "1" (see Fig. 1b) consists of fragment "3" with a further extension of about 50-60 nucleotides.

tides. It did not contain any unique sequences found in fragment "4", however, so that it is likely that position 77 of fragment "4" is at least 200 nucleotides away from the 5'-terminus of the molecule. We also estimate that position 77 of fragment "4" is at least 750 nucleotides away from the 3'-terminus of the molecule. This estimate is based on our studies of large fragments of the 16S RNA derived from a region protected against enzymatic digestion by enclosure within the 30S ribosomal subunit⁶. It is found that fragments "200" and "5", which largely overlap with fragment "4", also contain the sequence GCCUAAUACG, which is present only once in the molecule³ and is also found in fragments "sub-2" and "sub-3". Preliminary analysis of fragment "2", which is derived from milder conditions of partial digestion (Fig. 1b), has revealed that it encompasses all of section A, and additionally contains the further unique sequences GUCUAAUACUUUG and GCCUCUUG which are also present in the fragments from the subunit. The larger fragment from the subunit ("sub-2") contains 600 nucleotides, and does not contain the 3'-terminus of the molecule. Our findings with regard to the large fragments from the subunit have also enabled

us to determine the positions of the completed sections relative to each other in the molecule. All these relationships are diagrammatically illustrated in Fig. 4.

It is interesting that one of the rare clusters of methyl groups in the 16S RNA^{4,9} is found in the loop at the proposed point of folding of the molecule, and we believe that they must play some significant functional part.

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On the Possible Existence of Heavy Leptons

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Muon-pion invariant mass distributions ($M_{\mu\pi}$) observed in neutrino interactions and in the decays of the K_L^0 are compatible with the possible existence of a neutral lepton with a mass in the range $0.422 < M_{\mu\pi} < 0.437$ GeV.

IN neutrino experiments in 1967 (ref. 1), we used a 1.1 m³ heavy-liquid bubble chamber (HLBC) filled with propane (C₃H₈). In this liquid it is possible to determine invariant masses with greater precision than in the freon previously used. These conditions enabled me to continue a search, originally provoked by a study of the first neutrino experiments in the HLBC, for particles which decay into muons and pions.

Results from Neutrino Experiments

The ν interactions for the studies reported here have been selected according to criteria which ensure that they are produced by neutral primary particles; those interactions in which the resultant momentum in the neutrino direction is less than 0.3 GeV/c have been discarded. There are 251 ν events (μ^- , π^+) and 12 $\bar{\nu}$ events (μ^+ , π^-) which have a single possible combination of muon and identified pion of opposite charges for the calculation of

their invariant mass ($M_{\mu\pi}$). These data are shown in Fig. 1a in an ideogram in which the $M_{\mu\pi}$ distribution has been added with the assumption of a Gaussian distribution of the measurement errors. In the region of the largest enhancement in $M_{\mu\pi}$ the mean value of the measurement errors is ± 0.012 GeV. The most frequent source of the events contributing to the $M_{\mu\pi}$ distribution is from N^{*++} (1236) production in the interaction: $\nu_\mu + p \rightarrow \mu^- + \pi^+ + p$, either on a free proton or on a nucleon bound in carbon in C₃H₈. More than 80 per cent of the free proton events have $1.125 < M^* < 1.350$ GeV, where M^* is the "missing mass" of the hadronic system, calculated from the total visible energy of the event, the momentum of the muon, and its direction relative to the incident neutrino.

If the events in this range in M^* are removed from the data, the remaining 127 ν events and 6 $\bar{\nu}$ events give the $M_{\mu\pi}$ distribution in Fig. 1b. No theoretical phase space

is known for this distribution; a least squares fitted fourth-degree polynomial mean is compatible with an approximately uniform distribution. It can be seen that twelve of the $M_{\mu\pi}$ values in the largest peak in the histogram in Fig. 1a are due to the events of Fig. 1b. The $M_{\mu\pi}$ distribution of the difference between Fig. 1a and Fig. 1c contains six of the original eighteen values in this region of $M_{\mu\pi}$, with a mean of three events per bin. Because the statistical significance of the enhancement in the residue is less than that at the same value of $M_{\mu\pi}$ in Fig. 1c, the largest enhancement in Fig. 1a is unlikely to be due to N^{*++} (1236) production.

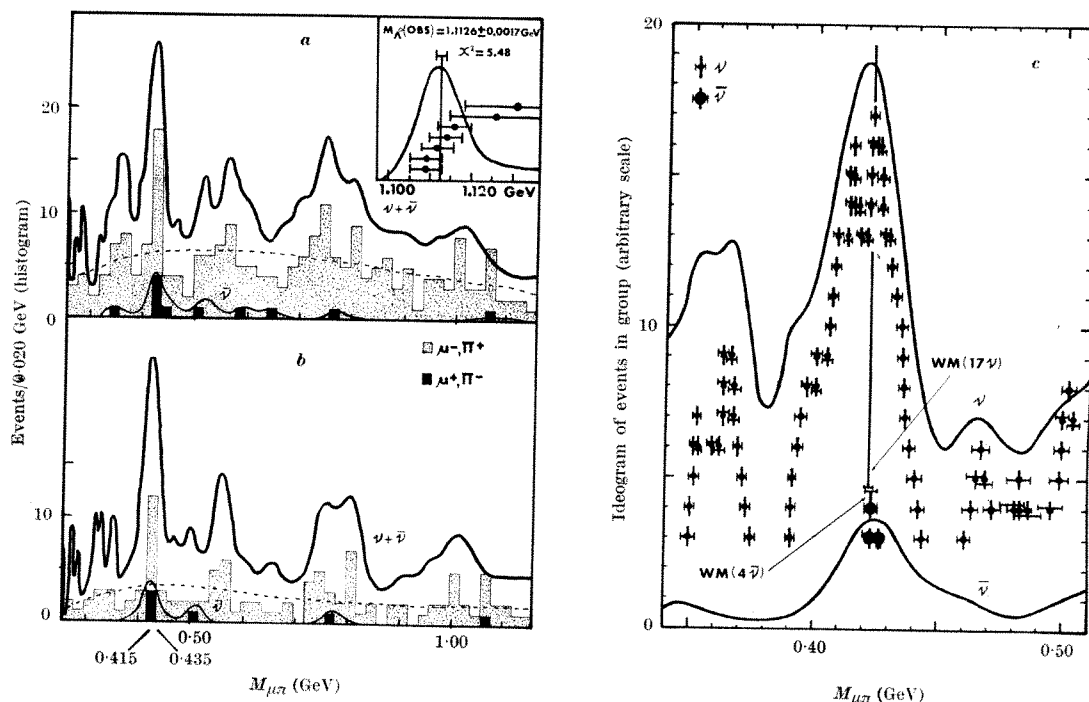


Fig. 1. Ideograms, histograms, and least squares fitted means for the $M_{\mu\pi}$ distribution for events giving one possible single muon-pion combination in the 1967 neutrino experiment. *a*, Total distribution for 217 ν events and 12 $\bar{\nu}$ events, the latter also shown separately (34 ν events are off scale). The inset shows the mass distribution of the seven Λ^0 obtained in the experiment. *b*, Distribution for 106 ν events and 6 $\bar{\nu}$ events remaining after selecting out events with $1.125 < M^* < 1.350$ GeV (21 ν events are off scale). *c*, Ideograms of all ν events and all $\bar{\nu}$ events in the region of $M_{\mu\pi} = 0.425$ GeV. The weighted means and their standard errors are shown for all the largest groups of three or more consecutive values of $M_{\mu\pi}$ with a confidence level ≥ 50 per cent. The two groups indicated are: WM (17 ν) = 0.423 ± 0.002 GeV; WM (4 $\bar{\nu}$) = 0.424 ± 0.003 GeV.

Fig. 1a shows that the largest peaks in the separate ideograms of all the ν events and all the $\bar{\nu}$ events occur at about the same value of $M_{\mu\pi}$; this is shown in detail in Fig. 1c. In order to investigate this coincidence, the weighted mean (WM) of every possible group of three or more consecutive values of $M_{\mu\pi}$ has been determined. The number of values in the largest group of each of such sets of groups which are compatible with their WM to a confidence level ≥ 50 per cent is also shown in Fig. 1c. Within the standard error of the WM, the largest group of ν events has the same WM as the largest group of $\bar{\nu}$ events. For a confidence level ≥ 90 per cent, the largest group of consecutive values of $M_{\mu\pi}$ in the peak of the combined ideogram of Fig. 1a contains sixteen ν events and four $\bar{\nu}$ events and has WM (16 ν ,4 $\bar{\nu}$) = 0.424 ± 0.002 GeV. These twenty consecutive values of $M_{\mu\pi}$ from the total of 263, and which to a confidence level ≥ 90 per cent have a mass and error distribution compatible with $M_{\mu\pi} = 0.424$ GeV, come from events whose visible energy is in the range $0.54 < E < 3.25_{\text{vis}}$ GeV. In five of these events the pion has been produced in the forward hemisphere with respect to the line of flight of the c.m.s. of $M_{\mu\pi}$.

The most likely physical phenomenon which could produce the same value of $M_{\mu\pi}$ in ν events and $\bar{\nu}$ events

over such an energy range is the decay of a neutral particle into a muon and a pion. An alternative explanation might be a statistical fluctuation in the $M_{\mu\pi}$ distribution. As a model, two Poisson distributions have been assumed with the same mean values of events as in the $M_{\mu\pi}$ distributions and in which an enhancement equal to that observed in the ν distribution has a WM which coincides within the observed limits with the WM of the enhancement in the $\bar{\nu}$ distribution. The probability of such a statistical fluctuation compatible with the observations is $< 10^{-4}$; therefore, the examination of $M_{\mu\pi}$ distributions has been continued.

The mass scale in Figs. 1a, b and c was calculated from the constants of the HLBC and its magnetic field distribution, which ranges in intensity from 27 kG to 19 kG over the visible volume. A test of the calibration of this scale may be obtained from seven Λ^0 which are associated with the ν events and which therefore have a similar probable distribution of their vertices to the $M_{\mu\pi}$ events. Their observed mass distribution is inset in Fig. 1a, WM(7 Λ^0) = 1.113 ± 0.002 GeV. The mass and error distribution of these seven events has $\chi^2 = 5.48$ about this mean value and $\chi^2 = 8.17$ about the nominal value of 1.116 GeV. If it is assumed that the discrepancy between the nominal Λ^0 mass and WM(7 Λ^0) is due to an error in the determination of momenta from curvature measurements, then the $M_{\mu\pi}$ values in the region WM(16 ν ,4 $\bar{\nu}$) should be increased on the average by 0.011 GeV. A specific cause of the discrepancy in the Λ^0 mass is not known, so the limits to WM(16 ν ,4 $\bar{\nu}$) will be defined for further reference as $L = 0.424 - 0.002$ GeV and $H = 0.424 + 0.013$ GeV.

Results from $K_{\mu 3}^0$ Experiment (HLBC)

Neutral muon-pion combinations with invariant masses in the range $L < M_{\mu\pi} < H$ occur in the $K_{\mu 3}^0$ decay. Such data, made available by H. W. K. Hopkins and K. J.

Peach, were obtained in an experiment² in which a beam of K_L^0 traversed a vacuum pipe through the HLBC. Owing to the 11 cm radiation length of the freon (CF_3Br), the $K_{\mu 3}^0$ decay can be separated reliably from $K_{\pi 3}^0$ and $K_{\pi 2}^0$ decays; however, pions are often indistinguishable from muons. No phenomenon was recorded which was not directly attributable to the K_L^0 decay. The mass scale was calibrated by a determination of the $K_{\pi 3}^0$ mass from 1,500 events with the same spatial distribution of vertices as for the $K_{\mu 3}^0$ events. In the range $L < M_{\mu\pi} < H$, the corrections to be applied to the mean mass scale are less than ± 0.001 GeV; the half-width of the $K_{\pi 3}^0$ mass distribution is ± 0.019 GeV.

In order to obtain the maximum information in the region $L < M_{\mu\pi} < H$, Fig. 2a was made from all events for which the reconstruction of the decay point in the vacuum pipe was possible. If calculable, both muon-pion assignments of the pairs of tracks were entered, irrespective of whether the correct assignment was known; there are 2,869 values in the range $0.250 < M_{\mu\pi} < 0.500$ GeV. A Monte Carlo calculation by Peach (to be published) for the $K_{\mu 3}^0$ decay, including both assignments and taking into account the effects of the vacuum pipe, gives the distribution shown in the range $0.380 < M_{\mu\pi} < 0.500$ GeV in Fig. 2a. In the region $L < M_{\mu\pi} < H$ the Monte Carlo curve and the least squares fitted polynomial means of the data from the fourth to the twelfth degree almost coincide. The variation of the confidence level of the fit of the data to the fourth degree mean is shown in Fig. 2b for all possible sets of seven, eleven and twenty-one consecutive bins; seven bins cover approximately the range in $M_{\mu\pi}$ between L and H . Each point in the diagram indicates the confidence level of χ^2 about the mean curve when the central bin of the set is at that value of $M_{\mu\pi}$. Irrespective of the choice of the number of consecutive bins in a set, the worst fit of the data to the mean is in the range $L < M_{\mu\pi} < H$. For the 100 bins in the data, $\chi^2 = 108.7$ to the fitted mean for 95 degrees of freedom.

Thus it is evident that the part of the observed $M_{\mu\pi}$ distribution which fluctuates most about the least squares fitted polynomial mean is in the region $L < M_{\mu\pi} < H$. There, however, such a mean coincides with the Monte Carlo calculation. In order to examine whether the fluctuations of the data about the mean, and therefore the Monte Carlo calculation, are likely to be of a statistical origin, a test has been made as follows: Let p_1 be defined as the higher momentum of the pair of tracks of an $M_{\mu\pi}$ combination and p_2 the lower. The data of Fig. 2a may then be divided into the two histograms with $p_\mu = p_1$ and $p_\pi = p_1$. Except when $p_1 = p_2$, $M_{\mu\pi}(p_\mu = p_1)$ is different from $M_{\mu\pi}(p_\pi = p_1)$. A real structure in the $M_{\mu\pi}$ distribution would be expected to be shown in each of the two samples; there is no *a priori* reason to expect a statistical fluctuation to give the same distribution in each sample.

The coefficient* X^2 has been calculated for the two sets of data derived from Fig. 2a; for the two sets of 11 bins in the range $0.415 < M_{\mu\pi} < 0.4425$ GeV, $X^2 = 3.57$. Thus over this range the two samples of data represent the same $M_{\mu\pi}$ distribution with a confidence of 96 per cent. A high confidence level is to be expected if the effect that causes the actual $M_{\mu\pi}$ distribution to differ from the Monte Carlo calculation is due to a physical phenomenon. An explanation of the effect in terms of a statistical fluctuation would imply that there must be two correlated statistical fluctuations which occur equally in both samples of

*In order to measure the correlation between the two histograms, the coefficient X^2 has been used.

$$X^2 = (N+M) \left[\frac{1}{M} \sum_{j=1}^k \frac{m_j^2}{m_j + n_j} + \frac{1}{N} \sum_{j=1}^k \frac{n_j^2}{m_j + n_j} - 1 \right]$$

where m_j and n_j are the contents of the j th bin of the two histograms, each with the same mass scale and containing N and M events, respectively. Asymptotically, X^2 becomes χ^2 for $(k-1)$ degrees of freedom.

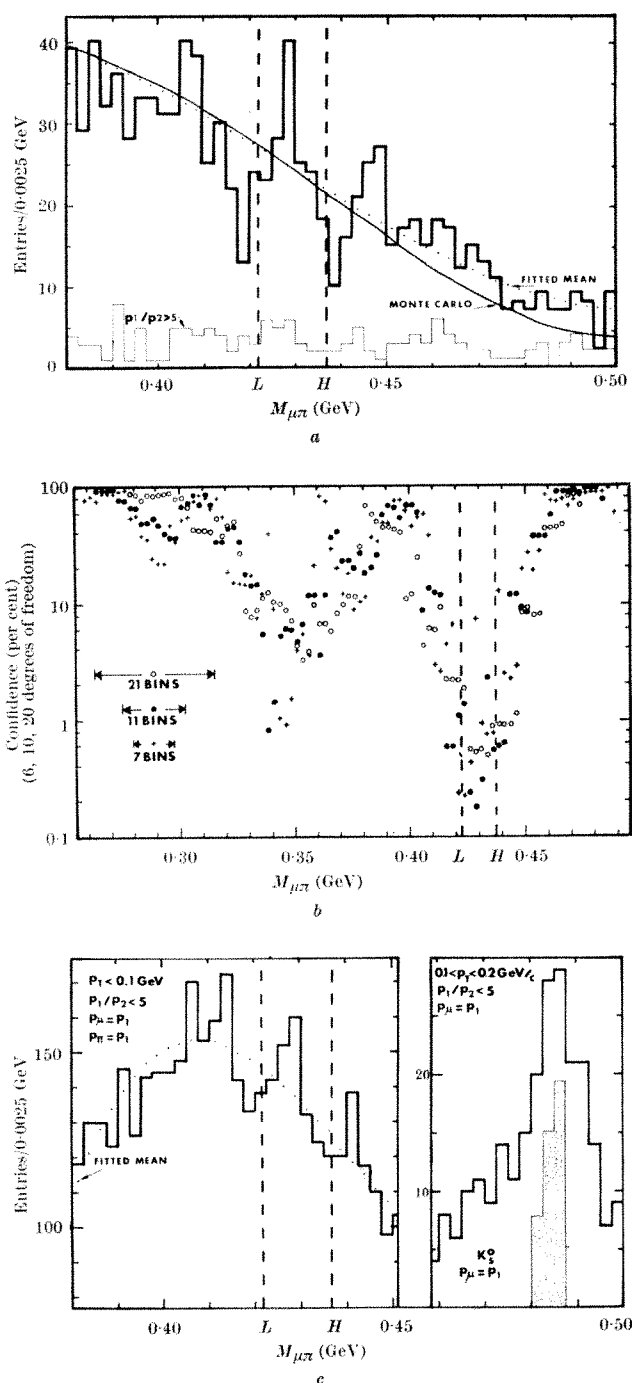


Fig. 2. Distributions of $M_{\mu\pi}$ from the decay of a K_L^0 beam traversing a vacuum pipe in the HLBC. *a*, Distribution from 2,869 values of $M_{\mu\pi}$ obtained from both possible assignments of each muon-pion combination. *b*, The confidence level of the fit to the mean of every set of seven, eleven and twenty-one bins. The value of $M_{\mu\pi}$ shown is for the central bin of the set. Distributions of $M_{\mu\pi}$ from 13,295 decays and interactions of K_L^0 in the HBC. *c*, In the range $0.380 < M_{\mu\pi} < 0.450$ GeV, both possible values are entered for the muon-pion assignment of all V-tracks. In the range $0.450 < M_{\mu\pi} < 0.500$ GeV, the shaded profile is the calculated histogram for the assignment of $K_L^0 \rightarrow \pi^+ + \pi^-$ as a muon-pion combination with $p_\mu = p_1$.

the data derived from Fig. 2a. It is estimated that the overall probability of the coincidence of a set of statistical fluctuations in the $M_{\mu\pi}$ distributions observed in the same bubble chamber in the range $L < M_{\mu\pi} < H$, and which would account for the effects indicated in Figs. 1c, 2a and 2b, is $< 10^{-7}$.

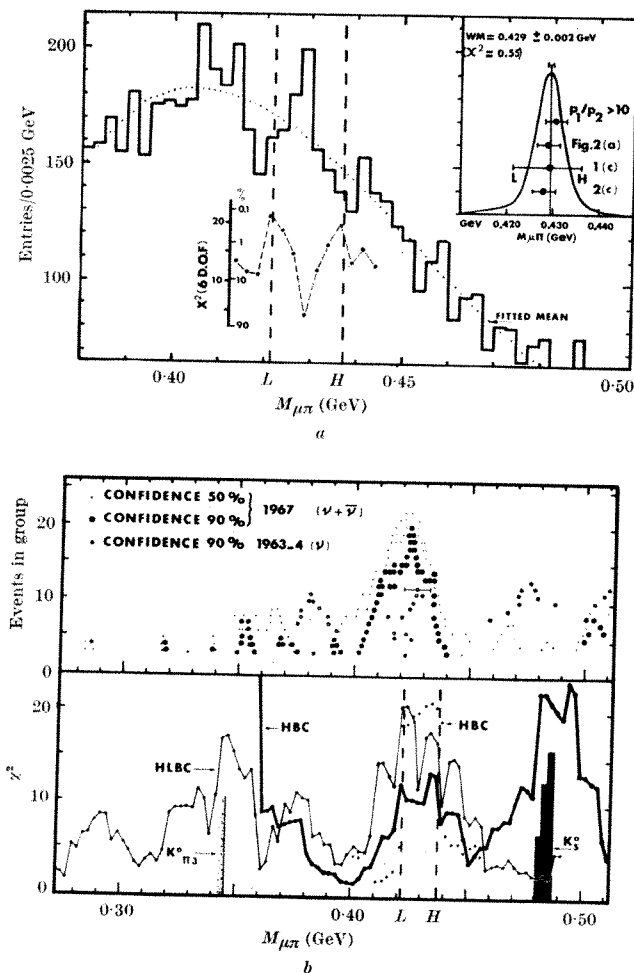


Fig. 3. *a*, $M_{\mu\pi}$ distribution from the combination of Fig. 2*a* and *c*. The broken curve shows the variation in χ^2 between the range $0.420 < M_{\mu\pi} < 0.4375$ GeV of Fig. 2*c* and neighbouring parts of Fig. 2*a*. Inset is the $M_{\mu\pi}$ distribution of the various phenomena in the range $L < M_{\mu\pi} < H$. *b*, The upper part of the diagram shows all the largest groups of three or more consecutive values of $M_{\mu\pi}$ from the $(\nu + \bar{\nu})$ events which have confidence levels ≥ 50 per cent and ≥ 90 per cent for their WM. The lower part shows the correspondence between the χ^2 distribution of every possible group of seven consecutive bins about the fitted mean in the $K^0_{\mu 3}$ data from the HLBC and HBC.

Results from $K^0_{\mu 3}$ Experiment (HBC)

Independent data³ on the $K^0_{\mu 3}$ decay in the Brookhaven 14 inch hydrogen bubble chamber (HBC) have been kindly made available by Hopkins. The observations consist of 13,295 V-tracks from the decays and interactions of a K^0_L beam, and are due to K^0_{e3} , $K^0_{\mu 3}$, $K^0_{\pi 3}$, K^0_s and Λ^0 . The masses of the particles producing the tracks cannot be determined: only the charges and momentum vectors are recorded: the $M_{\mu\pi}$ mass scale can be calibrated directly from K^0_s in the data assigned as $M_{\mu\pi}(p_\mu = p_1)$, as is shown in the right of Fig. 2*c*. For 85 per cent of K^0_s in the data $p_1/p_2 < 5$, this selection has been used to reduce the K^0_{e3} and Λ^0 contamination. The shaded profile is the expected histogram for K^0_s with the K^0_L spectrum, without measuring errors and assigned as $p_\mu = p_1$; the ordinate is proportional to the K^0_s flux. The $M_{\mu\pi}$ mass scale with $p_1/p_2 < 5$ is correct within ± 0.0025 GeV in the region of 0.485 GeV or ± 0.002 GeV near $L < M_{\mu\pi} < H$. Mass errors are only available for pion-pion assignments of the tracks; in the range $0.400 < M_{\mu\pi} < 0.450$ GeV the mean of the mass errors is ± 0.009 GeV.

In the range $L < M_{\mu\pi} < H$, 82 per cent of the $M_{\mu\pi}$ distribution from $K^0_{\mu 3}$ has $p_1/p_2 < 5$. The left side of Fig. 2*c* shows the $M_{\mu\pi}$ distribution from the HBC for $p_1/p_2 < 5$

and $p_T < 0.1$ GeV/c; the component $p_1/p_2 > 5$ for the HLBC data is shown in Fig. 2*a*: p_T is the observed momentum transverse to the direction of the K^0_L beam. Theoretically, the condition $p_T < 0.1$ GeV/c does not influence the distribution of correctly assigned $M_{\mu\pi}$ above 0.385 GeV. In Fig. 2*c* the unit of the ordinate has been scaled relatively to Fig. 2*a* by the ratio of the number of $K^0_{\mu 3}$ events in the HLBC ($\sim 1,500$) to the number of $K^0_{\mu 3}$ events in the HBC with $p_1/p_2 < 5$ ($\sim 3,400$). Thus, except for differences in experimental conditions, any physical structure in the $M_{\mu\pi}$ distribution of the $K^0_{\mu 3}$ would have the same amplitude in Fig. 2*a* and *c*. There is a similarity in the $M_{\mu\pi}$ distributions in and near $L < M_{\mu\pi} < H$.

The combination of the data in Figs. 2*a* and 2*c* is shown in Fig. 3*a*, as well as χ^2 between the seven bins in the range 0.420 GeV to 0.4375 GeV ($\sim L < M_{\mu\pi} < H$) of Fig. 2*c* and neighbouring parts of Fig. 2*a*. It is clear that the superposition of the two sets of data according to their mass scales gives the largest confidence level that each distribution in this range is the same. Thus it is observed that these $M_{\mu\pi}$ distributions, which have been obtained from K^0_L experiments in different laboratories and with different apparatus, have structures in the range $L < M_{\mu\pi} < H$ which correspond with a precision better than ± 0.0025 GeV. The variation of χ^2 for every possible set of seven consecutive bins about the least squares fitted mean for each set of data is shown in Fig. 3*b*; the points indicate χ^2 when the central bin of the set is at that value of $M_{\mu\pi}$. The HLBC data are those in Fig. 2*a*. For the HBC the component $p_\mu = p_1$ taken from the data in the left of Fig. 2*c* has been shown; this permits the best observation of the effects of backgrounds, which are not present to a significant level in the HLBC data. Both the location of the theoretical $M_{\mu\pi}$ distribution from the $p_\mu = p_1$ assignment of the K^0_s decays and the kinematical limit of the same assignment of the $K^0_{\pi 3}$ are shown. The χ^2 distribution of the sets of bins from the HBC data reflects both these backgrounds. In the region $L < M_{\mu\pi} < H$ there is an evident correspondence between the χ^2 distribution of the HLBC and HBC data, which is a further confirmation of the similarity of structure of the two $M_{\mu\pi}$ distributions. Also shown in Fig. 3*b* as individual points is part of the same type of χ^2 distribution for the $M_{\mu\pi}$ distribution of that part of the HBC data for which $p_1/p_2 > 10$. This sample, which has not been included elsewhere, shows an enhancement in the range $L < M_{\mu\pi} < H$ which has a probability $< 10^{-3}$ of being a statistical fluctuation.

The upper part of Fig. 3*b* shows the distribution of the WM of all the possible sets of consecutive values of $M_{\mu\pi}$ in the combined $(\nu + \bar{\nu})$ data of Fig. 1*a* which have a distribution in their values of $M_{\mu\pi}$, and their errors, compatible with the WM of the group to confidence levels of ≥ 50 per cent and ≥ 90 per cent. There are no other groups of events, within the range of $M_{\mu\pi}$ obtainable in $K^0_{\mu 3}$ decays, which are of similar size to those from which the range $L < M_{\mu\pi} < H$ has been defined. As already mentioned, the only group of $\bar{\nu}$ events is located in the same range. The WM of the groups with a confidence level ≥ 90 per cent, from the 1963-64 neutrino experiment, for which a smaller version of the HLBC was filled with CF_3Br , is also shown. It can be seen that in the region $L < M_{\mu\pi} < H$ these data are compatible with the 1967 results.

Possible Correlation in these Various Results

An explanation of these various observations in the range $L < M_{\mu\pi} < H$ as resulting entirely from a coincidence of statistical fluctuations seems sufficiently improbable to justify the consideration of other possible hypotheses. No known systematic errors in the analysis of the independent experiments, or backgrounds of other particles, are able to account for them. The particle already postulated as a possible cause of the effects in the neutrino data in the range $L < M_{\mu\pi} < H$ might also provide an

explanation of the effects in the K_L^0 data. In the neutrino interactions the postulated particle ($M_{\mu\pi}^*$) might be produced in a process such as: $\nu_\mu + N \rightarrow M_{\mu\pi}^* + N$, $M_{\mu\pi}^* \rightarrow \mu + \pi^+$. If the effects seen in the K_L^0 data originate in the $K_{\mu 3}^0$ decays, then a possible process could be: $K_L^0 \rightarrow \bar{\nu} + M_{\mu\pi}^*$, $M_{\mu\pi}^* \rightarrow \mu^- + \pi^+$. Analogous processes could be postulated for $\bar{M}_{\mu\pi}^*$. With such assumptions it might be expected that the peaks in the $M_{\mu\pi}$ distributions in the range $L < M_{\mu\pi} < H$ would occur at the value of $M_{\mu\pi}$ corresponding to the mass of the particle. The result of the calculation of a WM based on such an assumption is shown inset in Fig. 3a; for the neutrino experiment, the mass value $[(L+H)/2 \pm (L-H)/2]$ has been used. The four separate observations yield WM ($4M_{\mu\pi}^*$) = 0.429 ± 0.001 GeV, with a confidence level of 90 per cent; at this stage of the investigations, however, I shall continue to retain the range of mass of the possible particle as $L < M_{\mu\pi} < H$.

The observation of hadrons at the muon-pion vertex in the neutrino events implies that the postulated particle would have a lifetime that is too short for its decay path to be visible ($< 10^{-12}$ s), but sufficiently long to have an appreciable probability of escaping from the carbon nucleus ($> 10^{-22}$ s). Otherwise, by analogy with the N^{*++} (1236) production, the enhancement in Fig. 1c would be expected to extend over a greater range in mass, owing to scattering of the pion in the nucleus where it was produced⁴. A tentative estimate from Fig. 1a is that approximately 5 per cent of the ν events with a single muon-pion combination might be attributed to a process in which $M_{\mu\pi}^*$ is produced. Four out of the twelve $\bar{\nu}$ events might be attributed to the production of $\bar{M}_{\mu\pi}^*$.

An estimate of the partial branching ratio of the production of such a possible particle in the $K_{\mu 3}^0$ decay requires the postulate of a mechanism by which the fluctuations in the $M_{\mu\pi}$ distributions in the range $L < M_{\mu\pi} < H$ could be produced. It is observed that the fluctuations lie symmetrically about this range and about the mean. No plausible choice of a higher degree for the fitted polynomial mean has a significant effect on the χ^2 distribution in Fig. 3b. A part of such a type of structure in the $M_{\mu\pi}$ distribution of both possible assignments of the pairs of tracks from the $K_{\mu 3}^0$ decay might be attributable to a variation in the angular distribution of the muon-pion system in the K_L^0 decay in this region. This possibility is being studied; an isotropic decay of the muon-pion

system relative to the ν direction in the K_L^0 c.m.s. would require the implausible branching ratio of 11 ± 4 per cent in order to account for the excess of events in the range $L < M_{\mu\pi} < H$ with $p_1/p_2 > 10$. It has been noticed, however, that if the plane of the decay of the muon-pion system were preferentially parallel to the (K_L^0, ν) plane, a branching ratio of about 2 per cent would account for the excess of events with $p_1/p_2 > 10$. High values of p_1/p_2 occur more frequently in such a kinematical model.

Such considerations are, of course, speculative; my present aim has been to report the experimental observations which are compatible with the postulate of $M_{\mu\pi}^*$ so that they can be reinvestigated in other experiments and the postulate further tested. In this connexion, when more numerous data are available, other weaker enhancements in the $M_{\mu\pi}$ distribution might also be examined. It has been noticed that if they are numbered sequentially n , some fall close to the series: $M_{\mu\pi} = 0.250 \pm 0.005 n^2$ GeV. The possibility that there might be a charged lepton of this type might also be considered; P. G. Innocenti has drawn my attention to an enhancement in a muon-bremsstrahlung invariant mass distribution⁵ in the range $L < M_{\mu\pi} < H$.

The neutrino observations discussed in this report have been obtained by the combined efforts of many members of CERN, particularly my colleagues of the former Nuclear Physics Apparatus Division. I thank Robert Stump for many discussions about the early neutrino data, and H. W. K. Hopkins and K. J. Peach for their invaluable help in making it possible for me to examine the $K_{\mu 3}^0$ data. I also thank C. Grey-Morgan and G. C. Oades for their continued interest and criticisms and advice, and Mrs H. Cabel for assistance with the data analysis.

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Optical Polarization of the Crab Nebula Pulsar

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The optical polarization of this pulsar has been measured and compared with its radio polarization and the optical polarization of the surrounding nebula.

OF the radio observations of pulsars, polarization measurements are amongst the most revealing and, in particular, they have given the first direct evidence¹ for the rotation of these objects. A number of authors²⁻⁴, including ourselves⁵, have tried to measure the optical polarization properties of the Crab Nebula pulsar. Warner *et al.*² reported a variation of linear polarization across the pulses, and rotation of the polarization vector through them. We⁵ tentatively confirmed these results but also reported

variability of polarization on a time scale of minutes. We now believe this last report was incorrect and was based on an instrumental effect. Wampler *et al.*⁴, with a superior signal-to-noise ratio, confirmed the results of Warner *et al.* They reported, however, that they may have encountered thin clouds and, as they were using a single-channel polarimeter, they were forced to reduce their data in an approximate way which adversely affected their results.

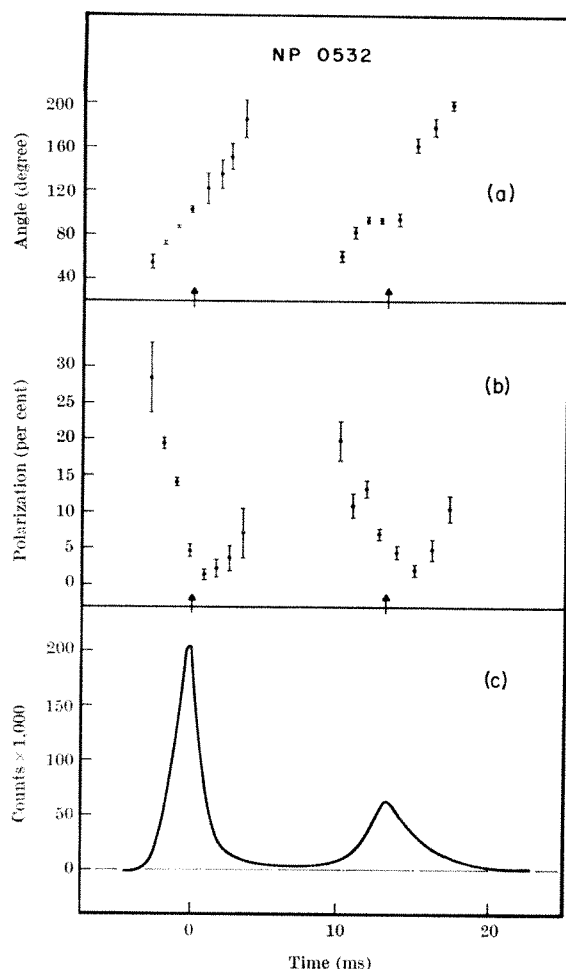


Fig. 1. The intrinsic linear polarizations of the pulsar in different parts of its light cycle. In all cases the horizontal axis is time relative to the main pulse peak. In *a* and *b*, arrows indicate the peaks of pulse intensity and probable errors are shown. In *c*, the photon noise is equivalent to less than the width of the line showing the pulse envelope, but the pulses are slightly broadened by timing resolution.

In our latest observations we find that the linear polarization in both pulses is high (~ 20 per cent) in the wings, but falls to virtually zero near the pulse centres, and that the polarization vector sweeps rapidly through a large angle ($\sim 140^\circ$) in both pulses. There does not seem to be any variation in either pulse-shape or polarization from night to night. When the interstellar polarization has been subtracted, the linear polarization is well defined and varies smoothly through the pulses in a manner which lends support to some sort of rotating model for the object. But the results do not take the simple form shown by the radio emissions of the Vela pulsar in which the intensity and percentage polarization follow one another closely¹. In the Crab pulsar the radio and optical polarizations differ markedly from one another. No obvious relation can be detected between the optical polarization of the pulsar and that of the Crab Nebula in its immediate environment.

Instrumentation

A two-channel polarimeter⁶ was used to eliminate the effects of changing transparency during observations. The outputs, after being amplified by two current-to-frequency convertors⁷, were cycled through two separate sets of channels in a multiscaler, at a rate consonant with the pulsar frequency (~ 30 Hz). The cycling was triggered by timing gear which consisted of a period generator whose base-resolution was $0.1 \mu\text{s}$ provided by a 10 MHz quartz crystal oven oscillator. The frequency stability

of the oscillator was continuously monitored against the 60 kHz radio timing signal transmitted by station WWVB.

Each output of the polarimeter occupied about 100 channels of the scaler, so each channel contains data from an interval within the pulse amounting to 3×10^{-4} s. By manual adjustment of the period generator during each run the effective timing resolution could be kept to 2×10^{-8} s which means that for each observing run of 200 s the total timing drift amounts to $200 \times 30 \times 2 \times 10^{-8}$ s, which is only a third of a channel wide. Timing precision of this order is important because the polarization varies so rapidly within the pulse.

Apart from improved timing precision the instrumental arrangement is similar to that used before⁵. Before these latest observations, however, it was discovered that the output of the two-channel current-to-frequency convertor, used in earlier observations, was modulated by the 60 Hz power line frequency. This had the effect of slightly broadening the pulsar pulses in such a way that the relative photometric accuracy over various parts of the light-curve was destroyed. Because the strength of this 60 Hz modulation was found to be variable, the anomalous results of our earlier observations are probably explained. Our report⁵ of variability of pulse-shape and polarization must therefore be withdrawn. In the observations reported here special care was taken to reduce the 60 Hz modulation to the point where it had no adverse effects.

Observational Procedure

Observations were made with detectors of S-13 response through a diaphragm of diameter 3 arc s, with no filter, at the Cassegrain ($f/9$) focus on the Steward 90-inch telescope, and were limited by weather to 75 minutes on the night of October 18, 1969, and 150 minutes on October 19. The polarimeter was rotated in the sequence 0° , 330° , 300° , 30° , 60° , 90° , 0° and so on. At each angle of the polarimeter three runs each of 200 s duration were made, two with the depolarizer out separated by one with it in. A total of fourteen such runs were made on the first night and twenty-four on the second. The data from the scaler were recorded on digital magnetic tape at the end of each run.

The data on the tapes were later reduced with the help of computers. Each data channel may contain light from both the pulsar and the nebular background. The light from 30 channels, 8.8 ms wide in all, centred at 13.25 ms ahead of the main pulse was assumed⁸ to be pure nebular background, and was averaged out and subtracted from all channels. The remaining light was assumed to be that from the pulsar. By combining the data in the usual manner^{6,9}, the polarization of the light in any one channel could be calculated. In practice, to improve photon statistics, the data from either three or four adjacent channels were combined into windows and the polarizations of the windows as a whole were computed.

The data from both nights separately indicated that the polarization near the main pulse peak was small (~ 2 per cent). Because of this, and because the pulse-intensity peak is extremely sharp¹⁰, the data from the two nights could be combined by superposing channels so that the pulse-peaks for the two nights coincided.

Zellner (personal communication) has shown the instrumental polarization of the telescope/polarimeter combination to amount to less than 0.16 per cent and it has been ignored.

Results

Each pulse was divided into eight windows, each window made up of three or four channels where one channel was 0.295 ms wide. The linear polarizations for both nights combined are given in Table 1 where all window positions are given with reference to the main pulse peak. The errors quoted are probable errors. The

polarization of the main pulse as a whole was found to be 6.5 ± 0.5 per cent at $96^\circ \pm 2^\circ$, and of the secondary 4.2 ± 0.4 per cent at $102^\circ \pm 2^\circ$. The polarization of the nebula seen through the same diaphragm was measured as 12.7 ± 0.5 per cent at $138^\circ.0 \pm 1.2^\circ$. The position of electric-vector maximum is measured from north, increasing positively toward the east. We note that with the integrated polarization of the main pulse being 6 per cent, a reduction procedure based on assuming it to be zero (as Wampler *et al.*⁴ did) will yield misleading results.

Table 1. LINEAR POLARIZATIONS FOR BOTH NIGHTS

Centre of window (ms)	Window width (channels)	Linear polarization (per cent)	Position angle (degrees)
Main pulse			
-3.25	3	26.5 ± 4.8	54 ± 5
-2.36	3	17.7 ± 0.8	74 ± 1
-1.48	3	13.2 ± 0.6	91 ± 1
-0.59	3	5.2 ± 0.8	114 ± 4
+0.44	3	1.6 ± 0.7	169 ± 14
+1.33	3	2.3 ± 1.2	116 ± 13
+2.21	3	4.0 ± 1.7	0 ± 12
+3.10	3	5.5 ± 3.4	36 ± 17
Secondary pulse			
+10.18	3	18.0 ± 2.7	61 ± 4
+11.06	3	9.9 ± 1.6	88 ± 5
+11.95	3	12.8 ± 1.1	99 ± 2
+12.83	3	6.9 ± 0.7	102 ± 3
+13.87	4	4.4 ± 0.9	108 ± 5
+15.05	4	3.9 ± 0.7	155 ± 6
+16.26	4	6.0 ± 1.4	171 ± 7
+17.40	4	10.2 ± 1.7	14 ± 4

With such low values of polarization near the pulse peaks, it is of interest to know what part of the measured polarization is interstellar and what intrinsic. We measured the polarization of ten stars fainter than twelfth magnitude within a few minutes of arc of the Crab. These yielded an interstellar polarization of 2.0 ± 0.2 per cent at $147^\circ \pm 3^\circ$. When the raw data of Table 1 are corrected for this we arrive at the intrinsic polarizations shown in Fig. 1. The main pulse as a whole is found to have a polarization of 7.2 ± 0.5 per cent at $88 \pm 2^\circ$ and the secondary 4.6 ± 0.4 per cent at $90 \pm 2^\circ.5$, while the corrected background value is 10.8 ± 0.5 per cent at $136^\circ \pm 1.2^\circ$. In Fig. 1 we notice how smoothly the polarization properties change through the pulses. The feature of high polarization in the wings with a low trough near the centre is common to both pulses, but there are considerable differences of behaviour in detail. The asymmetry of the polarization with respect to pulse intensity is also remarkable. In the main pulse the polarization trough follows the intensity peak by 0.55 ms, whereas for the secondary the interval is 1.6 ms. Thus the pulse peaks are separated by 41 per cent of a period and the polarization troughs by 44 per cent. In the main pulse the polarization falls rapidly then rises more slowly, while the converse is true for the secondary.

In the main pulse the polarization angle sweeps smoothly from front to back at 1.94° per degree of phase. The average rate for the secondary is 1.82 in the same units; the curious kink near the pulse peak could be due to noise. In the main pulse trough the polarization is 1.4 ± 0.7 per cent and in the secondary 2.0 ± 0.7 per cent.

Comparison of our results with the radio data is complicated by the differences in pulse shape between the radio and optical bands. Campbell *et al.*¹¹, working at 430 MHz, report that "the main pulse is about 18 per cent linearly polarized during its rise and its peak, and more highly polarized during its decay". They report the secondary as having the same general behaviour but with a lower peak polarization of less than 11 per cent. They also report no rotation of angle within the main pulse, although a rotation of 90° would have passed undetected.

So far as the comparison can be carried, there seem to be marked differences between the polarizations within the two bands. This confirms the suspicion, based on the shape of the total energy spectrum and the differing pulse profiles, that the emission mechanisms for the optical and radio radiation are different.

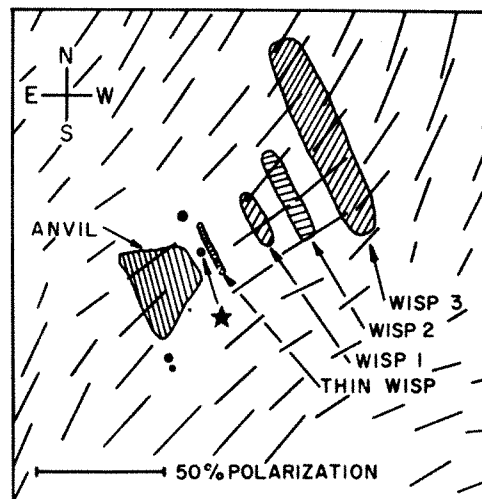


Fig. 2. Adopted from Scargle¹³. The central regions of the Crab Nebula showing prominent optical features. The solid black lines indicate the direction and amount of optical polarization measured by Woltjer¹⁴. The pulsar is marked with a star and, for scale, the star to the NE of it is 4 arc s away. In the nebula the length of the thin wisp corresponds to about 0.05 pc.

In view of the suggestion¹² that the pulsar provides the main energy source of the Crab nebula it is interesting to compare the optical polarization of the pulsar with that of the nebula. It can be seen in Fig. 2 that within 0.2 pc of the pulsar (projected on the sky) the polarization varies in amount between 10 and 25 per cent but the position of angle is fairly consistent and close to 140° (agreeing with our own background measurement very close to the pulsar). The wisps are at right-angles to this, parallel to the projected magnetic field (synchrotron model for the nebular continuum radiation). Comparing this figure of 140° with the optical polarization angles for the pulsar, no simple relationship can be discerned. The pulses as a whole are each polarized at about 90° . The intensity peaks are polarized at 110° and 95° for the main and secondary respectively. At the polarization troughs the angles are 122° (main) and 160° (secondary). The mean of these last two angles is close to 140° , but without a detailed model for the emission mechanism it is difficult to know what significance to attach to this coincidence.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Pulsar NP 0532: Polarization of Strong Pulses at 430 MHz as seen with 300 kHz Bandwidth

THE polarization properties of strong pulses from NP 0532 have been measured by Graham, Lyne and Smith¹, who used a 330 kHz bandwidth corresponding to a time resolution of about 2 ms; and by Heiles, Campbell and Rankin², who used a 10 kHz bandwidth corresponding to a time resolution of 120 μ s. The former authors measured large linear polarization; they did not know which pulse component was responsible for the strong pulses, but they suspected the precursor because of its high average linear polarization³. We measured systematic circular polarization in the strong main pulses, and proposed that the differences in results might be due to the difference in observing bandwidth.

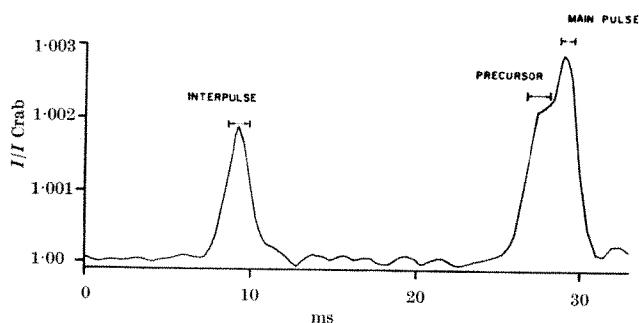


Fig. 1. Average of the total intensity, I , for 273,300 pulses. Also indicated are the phase intervals over which pulse energy was integrated to obtain the pulse energy distributions shown in Fig. 2.

We have repeated their experiment; we find the typical pulse to be less strongly polarized and show that strong pulses arise from the main pulse component. We used the Arecibo telescope and the 430 MHz dual circularly polarized line feed. All four Stokes parameters were measured simultaneously, two linear polarizations being generated by adding the two circular polarizations at our 30 MHz intermediate frequency with appropriate phase differences. The four signals were each passed through 300 kHz (to 3 dB points) filters, square-law detectors and time filters which were approximately rectangular of length 1 ms and were then digitized and recorded every 500 μ s. The effective time resolution of the dispersed pulses is slightly less than 2 ms. 273,300 pulses were

recorded on March 12, 1970. The average pulse shape in total intensity is shown in Fig. 1. The other average Stokes parameters were also obtained and agreed with our previous results³.

The criterion for selection of strong pulses was found from the distribution function of pulse energy for each of the three pulse components. The distribution functions for the precursor and the interpulse, however, showed no strong pulses whatsoever. Therefore, if these components occasionally emit strong pulses, they will be detected only at other frequencies or by frequency-sweeping techniques. On the other hand, strong main pulses are as obvious with this large bandwidth as they are with a small bandwidth²; the distribution function for main pulses is shown in Fig. 2. Of course, the fact that only strong main pulses exist shows that the strong pulse phenomenon is intrinsic to the source itself rather than to propagation effects, for example.

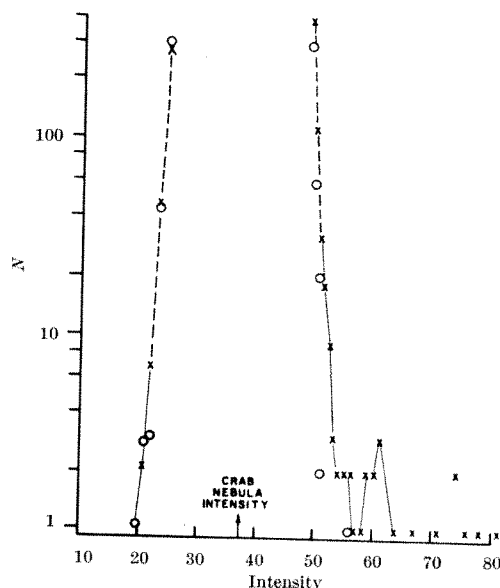


Fig. 2. Energy distributions for the main pulse (see Fig. 1) and for a comparison region of any pulse component. \times , On pulse; \circ , off pulse.

Stokes parameters for all of the strong main pulses were calculated and plotted. Most did not show the 70 per cent linear polarization implied to be typical by Graham, Lyne and Smith¹. Linear polarizations of order 25 per cent were typical but many pulses showed no polarization. There was no systematic trend in position angle from one pulse to another, except possibly as noted later. Circular polarization was nearly always smaller than linear polarization, usually less than 10 per cent, but definitely not always zero. A fairly typical strong pulse is shown in Fig. 3; the amount of linear polarization present here is typical, while circular polarization is somewhat stronger than usual.

The pulse numbers (with arbitrary zero) of the strong pulses are known to within ± 1 pulse and are given in Table 1. We were unable to find any nonrandomness in this sequence.

Table 1. STRONG PULSE NUMBERS

11928	65777	233297
13315	81170	251171
17448	pair {81337	{258910
27080	116732	pair {258930
37990	129436	pair {276145
41812	168909	{276571

We found three pairs of pulses separated by less than 500 pulses (about 15 s in time). The two members of each pair had similar polarization properties. One of

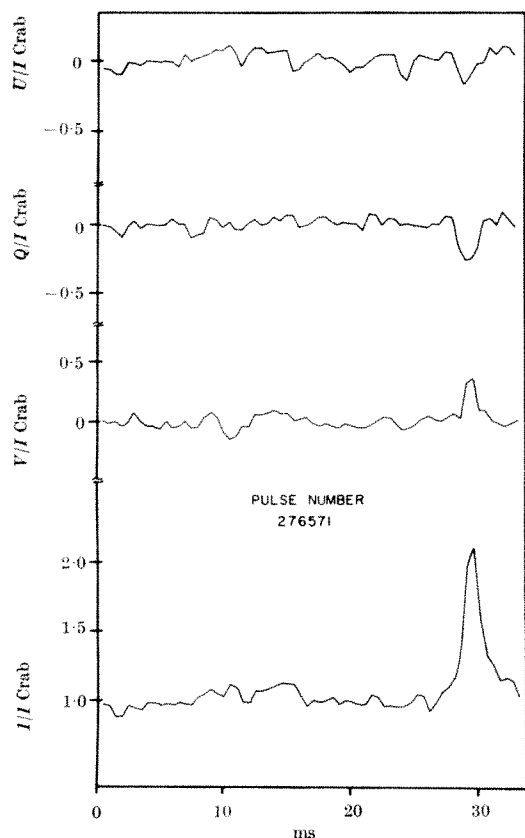


Fig. 3. Stokes parameters for pulse number 276571. This pulse has somewhat higher circular polarization than is typical, although its linear polarization is typical.

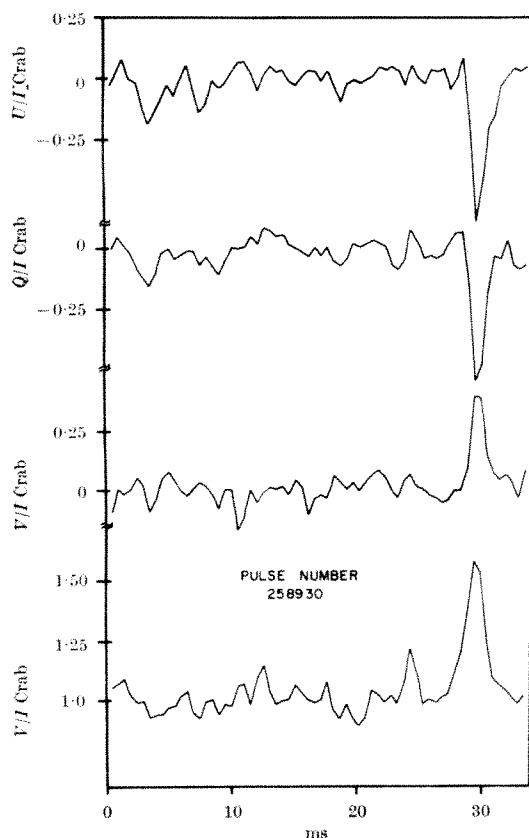


Fig. 4. The Stokes parameters for a pair of pulses. Both members of this pair were weak, but they are the most highly polarized of the strong pulses observed. The Stokes parameters for the two pulses behave similarly, as is also true for the other two pairs observed.

these pairs is shown in Fig. 3. The pulses in this pair are weaker than most of the other strong pulses, but are elliptically polarized and represent the most highly polarized strong pulses observed. The other pairs are more typical, showing weak polarization the sense of which is not related to that of other pulses.

Although we consider the number of pairs too small to draw a definite conclusion, the possibility is strong that this pairing effect is real because there is otherwise no systematic trend in the polarization properties. Credence is added to this possibility by the fact that the two most highly polarized pulses, shown in Fig. 4, are members of a pair. We shall report on this point in more detail in the future.

We thank D. C. Backer for use of some of his computer programs. The Arecibo Observatory is operated by Cornell University under contract to the National Science Foundation and with partial support from the Advanced Research Projects Agency. This work was supported in part by the Air Force Office of Scientific Research, Office of Aerospace Research, US Air Force.

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Correlation of the Amplitude of Successive Pulses of CP 0328

AN investigation of the variation in amplitude of individual pulses from pulsars was undertaken on June 29–31 using the 46 m antenna at Algonquin Radio Observatory with the receiver usually used in the long baseline interferometer programme. The system specifications were: right-hand circular polarization, a centre frequency of 408 MHz, r.f. bandwidth of 4 MHz, and a system noise of 300 K. The signals were detected, passed through a low-pass filter of variable bandwidth with the cut-off frequency set to 10 Hz for the observations reported here, and recorded on a high-speed chart recorder. Of the twenty-six pulsars investigated only CP 0328 had sufficiently intense pulses to warrant further analysis. A typical record is shown in Fig. 1.

Analysis consisted of determinations of the autocorrelation function and power spectrum for eight batches of 250 pulses. The results all had the same general appearance, and a representative batch is shown in Fig. 2a. Fig. 2b shows a similar analysis carried out on a batch of 250 random numbers used to simulate random intensity variations of a pulsar. In most respects the pulsar and random number data are very similar. In particular we see no evidence for any significant second periodicity in the pulsar data although this does not contradict the results of Taylor, Jura and Huguenin¹ because they show that second periodicities tend to become less pronounced at higher radio frequencies. The one significant difference is that the pulsar data show high correlation at a shift of one pulse period whereas the random numbers, as expected, show insignificant correlation. The correlation coefficient from all our data, shifted by one pulse

CP 0328

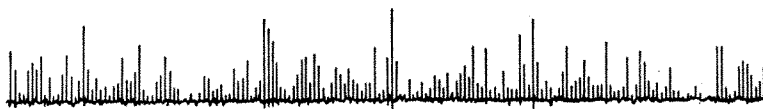


Fig. 1. A train of successive pulses from CP 0328 which is typical of the data used in our analysis.

period, is $P = 0.32$ which is significant at a level of $< 10^{-3}$. At a shift of two or more pulse periods the correlation is insignificant.

Several explanations of this correlation in the amplitude of adjacent pulses are possible. First, we can rule out instrumental effects. There is nothing in the receiving equipment that would store a significant fraction of the amplitude of a pulse for a time like the period of the pulsar which was 0.71 s. Fluctuations in the path from the pulsar to the observer would tend to give rise to pulse amplitude fluctuations on quite different time scales from that of the variation observed².

We can propose two models that refer to conditions near the pulsar (assuming the oblique rotator model). (1) The correlation of the radio pulses is due to an intrinsic modulation of the (otherwise random) flux of relativistic particles that give rise to the radio emission. (2) The correlation is due to the fact that the same particles contribute to the radiation seen in successive pulses.

These models can only be distinguished by further observations of several other pulsars. Results of a similar nature for PSR 0833-45 at 408 MHz by Jones and Wielebinski³ show that the form of the autocorrelation function

is quite different to that of CP 0328. Although the magnitude of the autocorrelation function is similar for a shift of one pulse period and is ≈ 0.4 , it remains significant for approximately twenty periods. This observation would tend to support model (1). If, however, model (2) is correct or partially correct there are some interesting consequences. If the radio frequency radiation is assumed to be emitted from within the light cylinder, because the extension of the particle stream responsible for the radio emission (which is assumed to be rotating with the pulsar) has to be greater than cP (where P is the pulsar period) for amplitude correlation of successive pulses to occur, and because the radius of the light cylinder is $cP/2\pi$, the angle between the axis of rotation and the magnetic field axis cannot exceed

$$\theta \approx \frac{cP/2\pi}{cP} = \frac{1}{2\pi} \approx 10^\circ$$

This analysis assumes the radiating particles' travel outwards from the magnetic poles in straight lines at approximately the speed of light. It seems clear that further observations of a similar nature on several

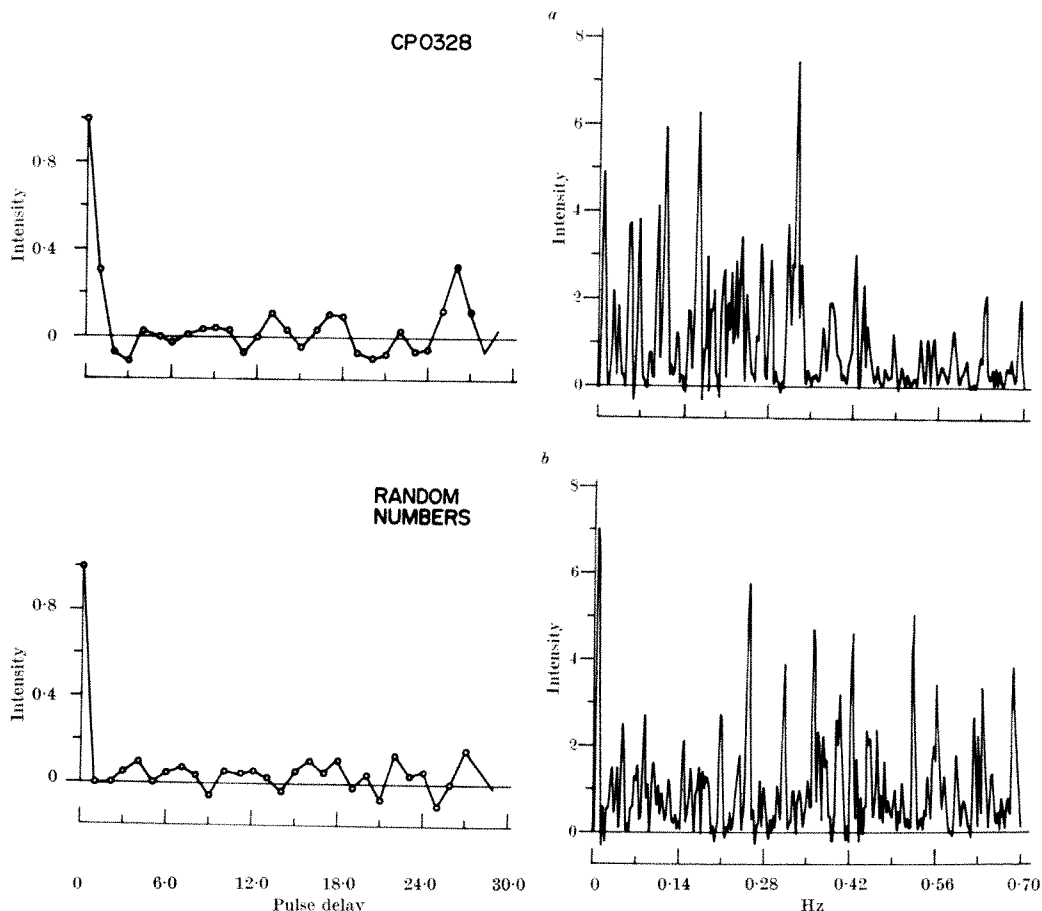


Fig. 2. The normalized autocorrelation functions and power spectra for 250 pulses. *a*, The result for CP 0328; *b*, the result from an analysis of random numbers. In the case of the autocorrelation functions, only the first thirty points out of a total of 249 have been shown for clarity.

pulsars would be of great value for attempts to evaluate the details of the radiation mechanism.

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Narrow-band Infrared Photometry of α Ori

BROAD-BAND infrared photometry of cool giants and supergiants shows that many of these stars have appreciable excess emission at 10 and 22 μ m (N and Q magnitudes brighter than expected from the temperatures deduced from shorter wavelengths). Gillett, Low and Stein¹ showed that the 10 μ m excess in several stars, including α Ori (M5Ia), was confined to a band peaking at about 10 μ m. Woolf and Ney² suggested that this emission peak corresponds to a peak in the emittance of silicate materials and that circumstellar dust composed of silicate produces the excess emission by thermal re-radiation of starlight. In order to provide an additional experimental test of this hypothesis we have made a series of narrow-band photometric observations of the star α Ori, extending out to 24.5 μ m. The spectral emittance of silicates is known to have a second peak near 20 μ m which should be even more diagnostic than the 10 μ m peak.

The observations were made with our standard multi-band photometer to which we added four additional interference filters. Mars was used as a calibration source

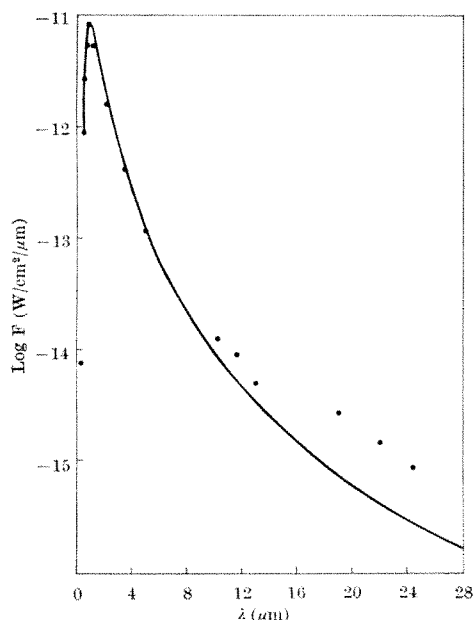


Fig. 1. Observed fluxes for the star α Ori are compared with the black body curve for $T = 3,500$ K.

outside the Earth's atmosphere and was found to be a good 240 K black body at 5, 10 and 22 μ m. These broad-band observations were based on our standard stellar calibration which is thought to be reliable to an absolute accuracy of ± 15 per cent (ref. 3).

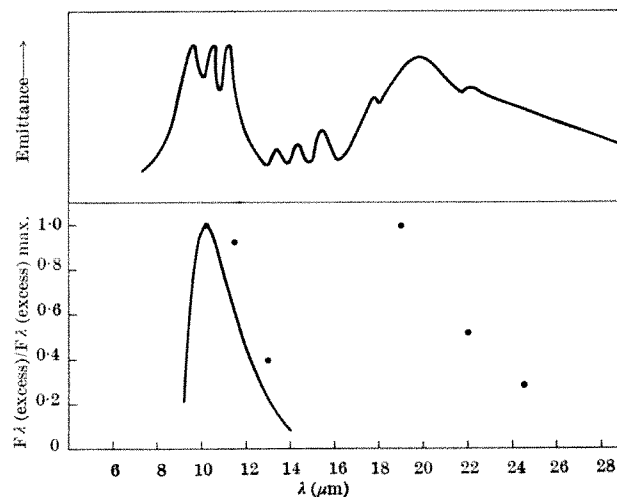


Fig. 2. Emission spectrum of orthopyroxene (top section) is compared with the observations of α Ori (dots) in the bottom section. The profile shown in the bottom section is obtained from the observations of Gillett *et al.*

The results are presented in Table 1 and Fig. 1, which include short wavelength data taken from unpublished work of Johnson *et al.* The solid curve in Fig. 1 shows the 3,500 K continuum of the star; the infrared excess is clearly seen. In Fig. 2 we have plotted the spectral emittance of silicate material taken from the work of Lyon (unpublished). Specifically, Lyon has measured the absorption spectrum of several hundred silicate rocks and minerals. From the family of curves given for orthopyroxene inosilicates we show as a typical case the composition chosen by Knacke *et al.*⁴. Below this curve we have plotted the 10–24.5 μ m excess fluxes obtained by subtracting out the extrapolated stellar continuum. The solid line represents the data of Gillett *et al.*¹, which are seen to be quite consistent with our new data, where they overlap. The reason that there are no data between 13 and 19 μ m is that the strong 15 μ m band of CO_2 obscures these wavelengths for ground-based observations.

Table 1. OBSERVED FLUXES FOR α ORI

Band pass	$\Delta\lambda$ (μ m)	Wavelength (μ m)	Flux ($\text{W cm}^{-2} \mu\text{m}^{-1}$)
U	—	0.36	7.6 (–14)
B	—	0.43	8.9 (–13)
V	—	0.54	2.7 (–12)
R	—	0.70	5.4 (–12)
I	—	0.90	8.3 (–12)
J	—	1.25	5.4 (–12)
K	—	2.20	1.6 (–12)
L	—	3.40	4.3 (–13)
M	1.0	5.00	1.2 (–13)
N	6.0	10.20	1.3 (–14)
O	1.0	11.50	9.5 (–15)
P	1.2	13.00	5.2 (–15)
Q	7.5	22.00	1.5 (–15)
R	1.0	19.00	2.8 (–15)
S	1.0	24.50	9.0 (–16)

The excellent agreement between the observed spectrum of silicate material and the observed spectrum of the infrared excess in α Ori is consistent with the silicate dust hypothesis.

The spectral emittance of silicate dust is quite insensitive to temperature from 100 to 500 K. The results of Fig. 2 are consistent with a temperature of 250 K. Using this temperature it is possible to calculate the minimum angular diameter of a unit emissivity disk capable of radiating the observed 22 μm flux density. This value, 0.7 arc s, is near the resolving power of present telescopes; it seems more likely, however, that the 22 μm angular diameter is ten or twenty times the minimum value and should be readily measurable. It can be seen from Fig. 1 that the total infrared excess is about 0.5 per cent of the total flux emitted by the star, which implies very low optical depth near one micron and suggests a large diffuse cloud. Our 22 μm photometry, however, was carried out with various beam sizes from 37 arc s down to 5 arc s in diameter and no decrease with decreasing beam size was found. Thus even the present observations place moderately severe limits on the size of the circumstellar dust cloud; a direct observational test of the thermal re-radiation model is now possible.

Assuming $T = 250$ K, a mean particle radius of 0.2 μm and a distance of 180 pc, the total number of grains producing the observed flux density at 22 μm is calculated to be $\sim 1 \times 10^{26}$ g.

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X-Ray Glow from Planetary Atmospheres

UNEXPECTEDLY high background X-radiation has been observed during a daytime stellar X-ray survey by rocket¹. The source of the radiation is attributed to X-ray fluorescence of the Earth's atmosphere. The results of calculations of the X-ray fluorescence spectrum which could be observed from a spacecraft near a planet are presented here. This spectrum should be a valuable tool in the determination of the composition of planetary atmospheres, particularly with regard to the minor constituents such as neon and argon on Venus and Mars and nitrogen and carbon on Jupiter. It could also be used to detect and identify the atmosphere of Mercury.

Table 1 lists the characteristic K_α emissions of elements which make up planetary atmospheres, together with the probability of K fluorescence ω_K (ref. 2). The number of photons $\text{cm}^{-2} \text{s}^{-1}$, I , which would arrive at a point distance Z above the surface of a planet is given by

$$I = \int_V \int_{\lambda_0}^{\lambda_K} \frac{n_K(Z) \sigma_K(\lambda) \omega_K}{4\pi R_s^2} Q_\infty(\lambda) e^{-\tau_1(\lambda, Z)} e^{-\tau_2(\lambda_K, Z, \theta)} d\lambda dV$$

where $n_K(Z)$ is the number density of emitting atoms, $\sigma_K(\lambda)$ is the ionization cross-section, ω_K is the probability of fluorescence, $Q_\infty(\lambda)$ is the incident solar X-ray

spectrum, $\tau_1(\lambda, Z)$ is the optical depth of the entering solar radiation and $\tau_2(\lambda_K, Z, \theta)$ the optical depth of the emitted radiation, and R_s is the distance between the emitting altitude and the observation point. The integration is performed over wavelength λ and emitting volume V encompassed by the total permissible solid angle.

Table 1. X-RAY FLUORESCENCE FROM PLANETARY ATMOSPHERES

Element	K_α (Å)	ω_K
C	44.54	0.0009
N	31.56	0.0015
O	23.57	0.0022
Ne	14.59	0.0081
Ar	4.19	0.12
Kr	0.98	0.66
Xe	0.42	0.88

Table 2 lists the results of the calculation at 4,000 km for different elements where the subsatellite point is the same as the subsolar point.

Table 2. EMISSION AT 4,000 KM (PHOTONS $\text{cm}^{-2} \text{s}^{-1}$)

Element	Mercury	Venus	Earth	Mars	Jupiter
C		3.4×10^3	8.7×10^{-1}	3.6×10^3	4.6×10^{-1}
N		3.2×10	8.4×10^2		9.8
O		2.2×10^4	2.7×10^2	2.4×10^3	
Ne	3.7×10^2	8.3×10^2		1.1×10^3	
Ar	2.8×10	6.9×10	6.4×10		

The results are limited by the great uncertainties in the atmospheric models. In the case of Mercury, two atmospheres were chosen, the first of neon with a surface pressure of 10^{-6} mb air and a constant scale height of 33 km, and the second of argon with the same scale height. For Venus three cases were considered in which 1 per cent argon, neon and nitrogen were added to the adopted model³. For Mars, the atmospheric model derived from the Mariner 6 and 7 radio occultation experiment was used⁴. In addition to CO_2 this model has as its principal constituent 10 per cent neon. Jupiter was considered although the chief constituents of its atmosphere are thought to be H_2 and He which do not show X-ray fluorescence. The adopted model⁵ has CH_4 as 5×10^{-3} and NH_3 as 10^{-3} of the He concentration. Any increases in these proportions would lead to larger C and N photon fluxes in direct proportion. The same holds true for the constituents of other planets. Because the solar spectrum chosen was representative of a quiet Sun, periods of solar activity will increase the fluorescence emission particularly below 30 Å. It should be noted that precipitation of energetic particles will contribute to the fluorescence as discussed by Tomlin⁶ for the Earth's auroral zone. Attempts to detect H_α emission from Jupiter have so far yielded negative results, however.

The flux behaves as $1/R_s^2$ and in most cases the constituents could be detected by instrumented spacecraft within 10^4 km of the planet. The flux expected at Earth orbit will on the average be 10^{-8} of that which appears at 4×10^8 km from the planet. This extremely small flux is probably less than the X-ray background, but if sophisticated instrumentation is available attempts should be made to detect a planetary X-ray flux.

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Dissolved Organic Carbon in the Equatorial Region of the Central Pacific

It has been reported¹⁻⁷ that the concentration of dissolved organic matter (DOM) in oceanic water is about 1 mg C/l. in the surface water and about 0.5 mg C/l. at great depths. Most of the DOM is considered to be supplied by decomposition of phytoplankton though in deep water DOM excreted by zooplankton may be significant. DOM is decomposed chiefly by microorganisms. It is assumed that the overall rate of DOM production is nearly balanced by the rate of decomposition and that fairly constant concentrations are maintained in the ocean.

During the north and equatorial Pacific cruise by the Hakuho-Maru from August 12 to November 13, 1969, I investigated the distribution of DOM in the Pacific on both sides of the equator.

The water samples were taken by Nansen bottles from nineteen stations between 50° N and 15° S at 155° W from depths ranging down to 5,340 m. One litre of each sample was filtered through a 47 mm diameter Whatman GF/C glass fibre filter precombusted at 500° C. The filtration was made immediately after sampling under vacuum of 185 mm Hg. Dissolved organic carbon (DOC) in the filtrate was determined in triplicate by the wet combustion method of Menzel and Vaccaro⁸. The precision of the determination was ± 0.05 mg C/l.

The distribution of DOC in the north-south section between 15° N and 15° S at 155° W longitude is shown in Fig. 1. The concentration of DOC was 0.8–1.4 mg C/l. in surface layers and it decreased to 0.4–0.7 mg C/l. in deep layers. These values generally agree with the results previously obtained in other regions of the oceans¹⁻⁷.

An interesting feature of my results is that the concentration of DOC was relatively low in the equatorial surface and subsurface waters (St-14, 0°, 155° W). Such a low concentration of DOC was probably caused by the Equatorial Undercurrent or Cromwell Current which has been described by Knauss^{9,10} and whose presence was confirmed by hydrographic recordings from the Hakuho-Maru.

At St-10 (10° N), vertical profiles of temperature, salinity, dissolved oxygen, nutrients and pH showed a clear upwelling movement of the surface water. In particular, dissolved oxygen was depleted in the sub-

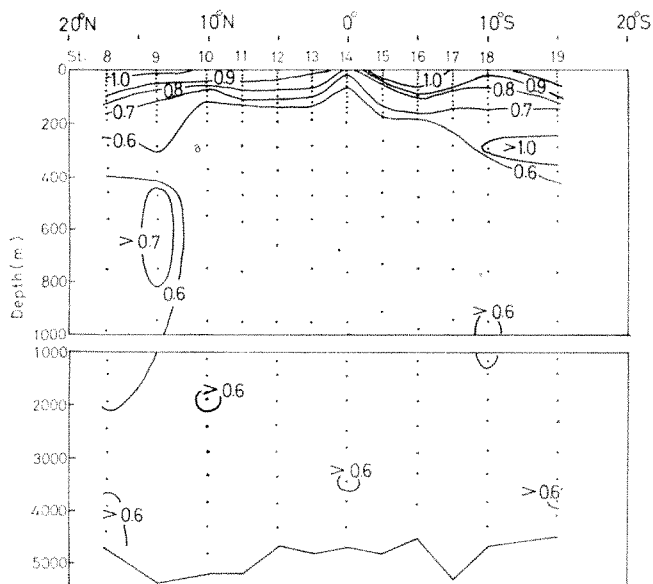


Fig. 1. Vertical profile of dissolved organic carbon (DOC) at 155° W (mg C/l.).

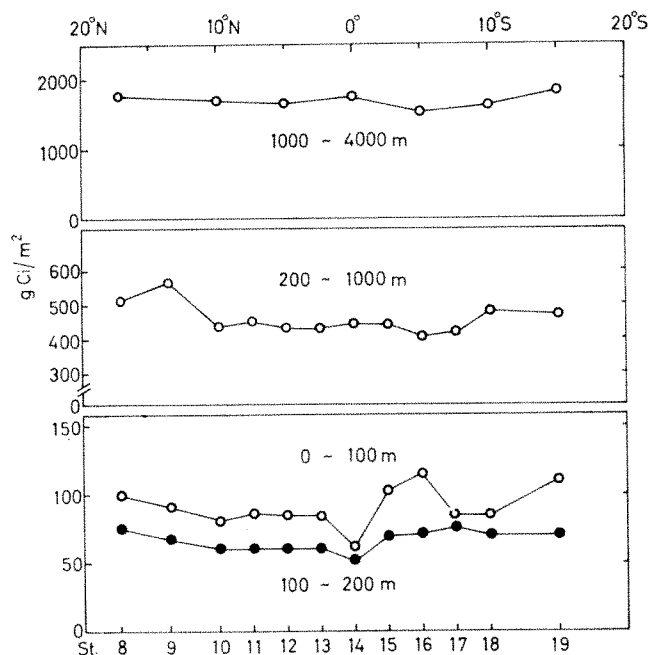


Fig. 2. Amount of DOC under 1 m² of sea surface (g C/m²).

surface layer and it was only 0.3 ml/l. at the depth of 150 m, but the very low concentration of DOC at the equator was not, however, found in the subsurface water of this station which suggests that the qualitative properties of DOM may be different.

Table 1. AMOUNT OF DOC UNDER 1 m² OF SEA SURFACE

Depth ranges	Location	(Number of station)	Mean $\pm a^*$	(g C/m²)
0-100 m	13.5° N-0°	(9-14)	81 ± 5	87 ± 5
	0°-15° S	(14-19)	93 ± 5	
100-200 m	13.5° N-0°	(9-14)	61 ± 5	65 ± 5
	0°-15° S	(14-19)	69 ± 5	
200-1,000 m	13.5° N-0°	(9-14)	460 ± 40	450 ± 40
	0°-15° S	(14-19)	440 ± 40	
1,000-4,000 m	17° N-0°	(8-14)	1,720 ± 150	1,710 ± 150
	0°-15° S	(14-19)	1,690 ± 150	
0-4,000 m	17° N-0°	(8-14)	2,320 ± 200	2,300 ± 200
	0°-15° S	(14-19)	2,290 ± 200	

*This value (a) indicates the ranges of analytical errors.

Average total amounts of DOC under 1 m² of sea surface are given for four depth ranges in Fig. 2 and Table 1. The average amount of DOC under 1 m² from the surface to a depth of 200 m was about 150 g, but at the equator the DOC content in the same water column was approximately 110 g, which is significantly lower than the average. It is noticeable that the DOC content in 0-200 m layer of the northern part of the equator was slightly higher than that of the southern part. Total amounts of DOC per 1 m² in the water column from the surface to a depth of 4,000 m at all stations fell within a narrow range of $2,300 \pm 200$ g.

This striking homogeneity in DOC distribution over the great depth range and width of the sampling area is surprising and interesting because carbon, being one of the typical bio-elements, is assumed to be easily subject to influences of biological activities. Similar homogeneous patterns of distribution of dissolved as well as particulate organic carbon have frequently been observed in other areas of the ocean and can be explained in terms of large scale oceanic circulation or biochemical inertness of organic carbon^{3,5}.

No apparent relationship between the concentration of DOC and chlorophyll *a* was observed in the surface layers, as I have shown previously⁷. Barber found low concentrations of DOC in the Cromwell Current (0°, 92° W)

and ascribed this to low primary productivity of the newly upwelled water¹¹, but my results suggest that low concentrations of DOC are not caused by low primary productivity, although the concentration of DOC may be low in the source water of the Equatorial Undercurrent.

DOM excreted by phytoplankton and zooplankton may be utilized by microorganisms soon after it has been excreted and this would probably obscure any clear-cut relation between the concentration of DOM and biological activities.

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Geomagnetic Reversals and the Position of the North Magnetic Pole

THE principal magnetic field of the Earth, often called the dipole field, is thought to be caused by a homogeneous dynamo action in the Earth's fluid core¹. The flow pattern in the core is not known, and there is even controversy as to whether it is in a state of turbulence² or whether the flow pattern is smooth and of a scale comparable with the circulation patterns in the atmosphere and oceans. If the flow is large scale and smooth it must be reasonably complicated, for the most simple flow patterns have too great a symmetry and have been shown definitely not to work as regenerative dynamos³⁻⁵.

Evidence is mounting that a further theorem prohibiting symmetry may exist, which is even more restrictive. Braginskii⁶ and Tough⁷ have derived it in limiting cases, and it is demonstrated by the theoretical dynamo of Herzenberg⁸. It would be that the streamlines of flow cannot have planes on either side of which they show mirror-image symmetry. Thus Herzenberg's dynamo fails to work when the axes of the two rotors are co-planar.

The original Bullard-Gellman dynamo⁹ perhaps suffered from this defect, in that further work demonstrated that the numerical process on which it depended did not converge¹⁰. My recent work¹¹ on a modification of the early Bullard-Gellman model shows that the dynamo may work when the planes of symmetry are destroyed. Fig. 1a shows the original $T_1S_2^2$ dynamo of Bullard and Gellman, with the planes of streamline symmetry marked in as A-A' and B-B', and Fig. 1b shows my recent $T_1S_2^2S_2^2$ dynamo, in which the two planes of symmetry have been destroyed. Nagata has pointed out¹² that this is perhaps a mechanism for geomagnetic reversals: the flow pattern, usually asymmetric, approaches symmetry and the dipole field decays. Though there has been no generalization of Braginskii's theorem, it nevertheless seems reasonable to expect at this time that the flow in the core has a certain asymmetry.

The great uncertainty of the core motions has been referred to, in spite of the progress made by downward

continuation of the observed secular variation¹³. There is, however, one fact on which nearly all dynamo workers are agreed: this is the importance of the Coriolis force, caused by the Earth's rotation, in controlling the core flow pattern¹⁴. The evidence for this is the coincidence of the geomagnetic dipole with the rotation axis, averaged over periods of geologic time, and tested by palaeo-magnetic studies.

The dipole field, controlled by the Coriolis forces, would therefore be expected to be symmetrical about the axis of rotation. When examined closely, of course, it is not exactly parallel: the present geomagnetic dipole deviates by $11\frac{1}{2}^\circ$ (approximately the co-latitude of the North magnetic pole).

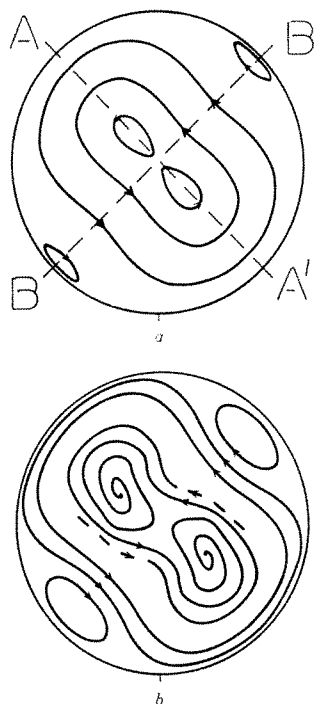


Fig. 1. Streamlines in the equatorial plane of a sphere. a, The $T_1S_2^2$ dynamo of Bullard and Gellman, with A-A' and B-B' marking the planes of symmetry of the flow pattern; b, the $T_1S_2^2S_2^2$ dynamo of Lilley, with the planes of symmetry destroyed.

The purpose of this note is to advance the hypothesis that this deviation of the geomagnetic axial dipole from true North, the expected axis of symmetry, is in fact an expression of asymmetric motion in the Earth's core. It follows that a wandering of the magnetic poles to coincide with the geographic poles may be an indication of the flow in the core becoming symmetrical. If this should happen dynamo action will be lost and the dipole field will decay, perhaps to grow again in the opposite direction (that is, reverse), when the flow pattern once more becomes asymmetric. As a corollary, strong dipole fields may accompany a large deviation of magnetic North from true North.

It may be possible to test this hypothesis using palaeo-magnetic data from secular variation studies, such as that of Doell and Cox¹⁵, and from studies of geomagnetic reversals. Not only would one look for small displacements of the magnetic from the geographic pole at times of reversals, but also the corollary of strong palaeo-fields coinciding with large displacements should be examined. Confirmation of the hypothesis would provide valuable information on the fundamental problem of the dynamo process in the core.

The recorded measurements of the geomagnetic field over historic time give some support to this idea. As discussed by Nagata¹⁶, the recent secular variation has consisted of a decrease in the strength of the dipole field of 0.05 per cent per year, accompanied by a rotation of the dipole towards the geographic axis of 0.02° of latitude per year. These figures are reasonable if a 100 per cent decrease were to accompany a 11½° rotation.

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Eclogites as Products of Thermal Metamorphism

STUDIES of reported eclogites associated with gabbroic rocks and recent experimental data suggest that eclogites can occur as products of thermal metamorphism. This observation seems evident when the descriptions of certain rock series are reviewed, but it is rather unexpected given the general context accorded to the concepts of the eclogite or eclogite facies. The mechanism which produces these eclogites is the intrusion of a basic magma into a series of rocks constrained at high pressures (> 6 Kbar).

The original definition of the term eclogite, and consequently the eclogite facies, given by Haüy¹ has been interpreted and modified many times. In the context of present petrographic knowledge and petrologic interpretation, the following definition will be used here: an eclogite is a rock composed primarily of omphacite (diopsidic clinopyroxene in which jadeite predominates over Tschermak's molecule) and almandine-pyropgrossular garnet^{2,3}. The most important petrographic criteria used to establish the existence of the eclogite facies is that omphacite and garnet are stable together in rocks of approximately basaltic composition.

A general definition of the pressure-temperature conditions under which the eclogite facies exists is given by Turner³ and more precisely by Velde *et al.*⁴ for lower temperatures, and O'Hara⁵ and Ringwood and Green⁶ for higher temperatures. The notable character of this facies is that it is separated from the amphibolite facies by a boundary of negative slope ($P=x, T=y$) and from the granulite facies by a positive slope (Fig. 1).

A number of petrographic studies report spatial relations between amphibolite, eclogite and granulite facies rocks and gabbros over short geographic distances. These studies can be divided into three groups.

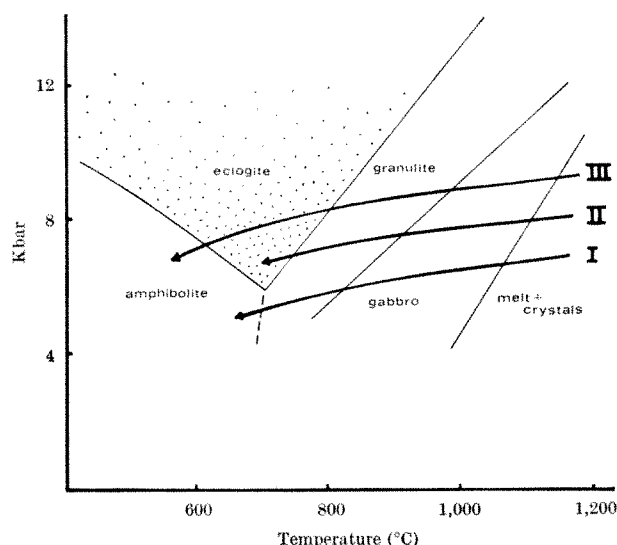


Fig. 1. Representation of the pressure-temperature limits of the eclogite facies and its relation to the amphibolite and granulite facies and the conditions of plutonism at high pressures. I, II, and III indicate the cooling path taken by magmas intruded at different pressure conditions as discussed in the text.

(1) Coronites or garnet hyperites⁷⁻⁹ show the transition between gabbro, granulite facies rocks and amphibolites. Typically, newly formed garnets are found as reaction rims between plagioclase and olivine or hypersthene grains, but the gabbroic texture of the rock is usually preserved in spite of the partial destruction of the original igneous mineralogy. The new assemblage is augitic pyroxene, garnet and plagioclase. This association is, in its turn, replaced by a garnet amphibolite rock at the outer margins of the basic bodies as they come in contact with a gneissic country rock. The thickness of the original plutonic rock units varies between metres and kilometres.

(2) A study by Vogel¹⁰ in northern Spain illustrates the transition between granulite and eclogite facies rocks. Mineral data show that the passage between granulite (garnet-clinopyroxene-plagioclase) and eclogite (garnet-omphacite) is accompanied by a change in pyroxene jadeite content from 6 per cent to 20 per cent while Tschermak's molecule content remains near 6 per cent. Relicts of gabbroic mineralogy and texture found in the granulite facies rocks demonstrate the plutonic origin of the massif.

(3) The transformation of gabbro to amphibolite through an intermediate granulite and eclogite stage has been described in France^{11,12} and in Japan¹³⁻¹⁵. The authors in the first case justify the eclogite terminology largely through the observed disappearance of plagioclase and the occasional presence of kyanite in essentially garnet-clinopyroxene rocks. The Japanese studies, however, indicate a decided increase in jadeite content in the clinopyroxene towards the exterior of a basic massif (Jd_0 to Jd_{10}). Each of the studies denotes a pronounced concentric aspect of the zones in the intrusive bodies. Frequently a gabbroic texture is preserved in the central parts, passing successively into garnet pyroxenite, eclogite and eventually amphibolite at the borders. Piboule and Coffrant¹² describe garnet or epidote amphibolites, and Shido¹⁵, Miyashiro¹⁴ and Seki and Banno¹³ a succession of first epidote amphibolites and then glaucophanites as the border assemblages. In each case the amphibolitized outer portion of the basic body is in the same metamorphic facies as the country rock.

Fig. 1 shows the probable pressure-temperature gradients under which the basic intrusives crystallized for the three cases I have discussed. The original intrusive material is considered to have been originally of basaltic composition. In all three examples the centre of the

intrusive represents a higher temperature of crystallization and equilibrium than the conditions of regional metamorphism.

Because the eclogite facies is found between the granulite and amphibolite facies above 6 Kbar, a cooling sequence above this pressure would bring a hot intrusive through the granulite, then the eclogite and, finally, the amphibolite facies as it attains equilibrium with the country rock. If this process is not completed for the intrusive, remnants of the sequence should be observed. Eclogites can therefore be the result of thermal metamorphism. In the instances I have discussed the intrusive mass gradually readjusts towards its exterior, to the conditions of regional metamorphism. The result could be aptly described as retrograde autometamorphism.

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Rubidium and Potassium Contents of Geosynclinal Basalts in the Japanese Islands

GREEN volcanics are frequently found in the Palaeozoic system of the Carboniferous and Permian in Japan. They are intercalated in sedimentary rocks of the eugeosynclinal facies laid down in the Chichibu (Honshu) geosyncline. The green volcanics in the geosyncline are mostly basaltic lava, sill and pyroclastic rocks. The presence of pillow lava implies that almost all of them were the product of submarine volcanism. We have examined the green volcanics from various parts of the Palaeozoic, and have made chemical analyses of both major and minor elements to determine their petrochemical features and to consider the environment of volcanic eruption. In this paper, we discuss the rubidium and potassium contents of lava and sill collected from western Japan, and their bearing on recent discoveries of petrochemical features of Cainozoic volcanics elsewhere¹⁻³.

The samples (about fifty) reported here were all altered in some way. To eliminate or minimize the effect of alteration, the following procedure was adopted: we analysed calcium and magnesium carbonates, and recalculated, after excluding the carbonate portion, each bulk chemical composition to 100 per cent on a carbonate-free basis. By this recalculation, K_2O increases at most by 0.1 per cent. The silica content for each of the samples is usually less than 55 per cent and falls within the range of basaltic composition.

The Palaeozoic geosynclinal basalts show remarkably low potassium contents and high K/Rb ratios. In the K/Rb-K diagram (Fig. 1), the basalts fall mostly in the region of ratios higher than 300. This high ratio is a

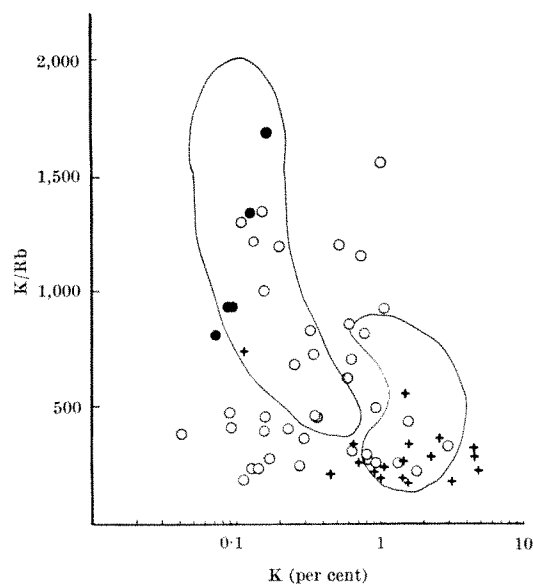


Fig. 1. Diagram showing the relation between K/Rb ratios and K contents of volcanic rocks in Japan. Circles, both opened and closed, are Palaeozoic geosynclinal basalts. Closed circles show the lowest ratio of K/Rb (the sensitivity of X-ray fluorescence analysis is 1 p.p.m. for Rb¹⁰; the ratio was therefore calculated assuming the Rb content to be 1 p.p.m. for these samples). Crosses are Cainozoic volcanic rocks^{5,6}. The left and right encircled areas correspond to the divisions of abyssal basalts and oceanic alkali basalts, respectively, which were given by Gast². A cross (K=0.133 per cent, Rb=727 p.p.m.) is excluded in calculating the average K/Rb ratio in the text.

marked feature, and the plot of Palaeozoic geosynclinal basalts is comparable with the range for abyssal and alkali basalts in oceanic regions¹. Other authors have discussed the range of the ratio¹⁻³, and have concluded that the ratio is a reliable criterion in distinguishing oceanic from continental volcanic rocks. On the other hand, the lower ratio may have resulted from alteration both during low grade metamorphism and during exchange with seawater⁴. The high ratio, regardless of any alteration, is therefore considered to be intrinsic.

According to the recently published data of the Japanese Cainozoic volcanics (of the continental margin)^{5,6} the K/Rb ratios for the Cainozoic volcanics average 266 which does not much exceed world-wide sources⁷. Hence, it becomes apparent that the ratios of the Palaeozoic geosynclinal basalts are much closer to those of oceanic basalts than those of the Japanese Cainozoic volcanics.

We conclude that Palaeozoic geosynclinal basalts are of a similar nature to basalts on oceanic ridges and oceanic islands. The Chichibu Geosyncline was formed presumably directly on oceanic crust or on fairly thin continental crust, because heavy pre-Carboniferous sediments have scarcely been found in the geosyncline. The magma which erupted into the geosyncline and produced basalts seems to have had little opportunity of reacting with the crust.

The geological significance of volcanic rocks in orogenic belts, which have been called ophiolite and spilite, may be summarized as follows. (1) Geosynclinal basalts like oceanic basalts were derived from magma through the oceanic crust and scarcely reacted with the continental crust, or the basalts might have been a part of oceanic crust. The basalts would therefore provide important information on the process of magma generation and the composition of the mantle. (2) While the traceable history of magmatism in oceanic regions is limited to the past 1.5×10^8 years, as suggested by the ocean floor spreading hypothesis, that of geosynclines exceeds this period in age. As a result, it may be possible by analysis of geosynclinal basalts to trace back the magmatic history in oceanic regions further into the pre-Mesozoic age. The hypothesis of ocean driven plate mechanics recently

proposed by Dewey and others^{8,9} suggests that slices of older oceanic crust are preserved as ophiolite suite in the orogenic belt. The petrochemical evidences of Japanese Palaeozoic basalts strongly support this suggestion. Geosynclinal basalts might therefore be called "fossils of volcanic rocks in oceanic regions".

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Upper Karroo Pillow Lava and a New Sauropod Horizon in Rhodesia

LAKE Kariba was maintained at full storage level, 1,600 feet above mean sea level, for several months in 1969, and has since been drawn down to around 1,585 feet. Wave action during the period of high lake level caused considerable erosion around exposed coasts on the lake shore. At the lower level, almost continuous exposure of rock is available in parts of the Zambezi valley where natural exposures were previously poor, and it is now possible to see details of the geology hitherto hidden. This is particularly noticeable in those parts of the lake where the solid geology consists of the lavas and interbedded sediments belonging to the Stormberg (Lower Jurassic) stage of the Karroo System. Cliffs on the islands just west of the Bumi estuary (centred on lat. 16° 47' S, long. 28° 13' E) have proved particularly revealing.

The first lava flow of the Stormberg is succeeded by about 100 feet of coarse gritty current bedded sandstone, from near the top of which the pelvis, sacrum, hind leg, tarsus, foot and parts of the tail of a large sauropod, together with some associated teeth, have recently been excavated from one of the new cliffs. The femur as preserved, lacking the distal end, measures 960 mm in length. Because Lower Jurassic dinosaur remains are rare¹, this articulated partial skeleton is of great interest. The new fossil horizon lies stratigraphically not far above the prosauropod-bearing Forest Sandstone of Upper Triassic age^{2,3}. There is a much greater gap between it and the recently discovered Gokwe⁴ and the Kads formations⁵. The latter contains the bones of reptiles comparable in size with *Brachiosaurus*⁶, and is probably of late Jurassic age.

The new fossil horizon is overlain by a series of basalt lavas and thin interbedded sandstones. The base of the next flow in the succession is exposed in a cliff section 6 miles west-south-west of the fossil locality. The basalt is of a type unusual in the Stormberg and contains feldspar phenocrysts more than an inch in length. The cliff shows that the base of this flow has beautifully developed pillow



Fig. 1. Pillow base of basalt flow resting on Karroo sandstones, Lake Kariba, Rhodesia. The pale rim of the pillows is the glassy skin, and the areas between the pillows contain sediment and shards of basaltic glass.

structures, the pillows being from 2 to 5 feet thick, and there are two or three layers of pillows, with sediment in the pockets between them. The flow is about 60 feet thick, with a massive central part and a vesicular top. The sandstones between the next few flows show soft sediment deformation at their contacts with the lavas, but pillow structures are confined to the base of the porphyritic flow, and even there they are only developed locally.

These observations have a three-fold significance. So far as we are aware this is the first recorded instance of pillow lavas in the Stromberg succession of Southern Africa. The sauropod remains are from a horizon from which no others have been recovered from this region; indeed, sauropods are extremely rare anywhere in rocks of this age. The presence of pillow lavas, sauropods and subaqueous sediments indicates the existence in this area of an "oasis" facies in rocks deposited at a time when desert conditions prevailed over much of this part of Gondwanaland.

A full account of these observations is being prepared for publication elsewhere.

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Some Ordering of Iron and Magnesium at the Octahedrally Coordinated Sites in a Magnesium-rich Olivine

FERROUS ions in crystal structures of silicates frequently prefer octahedrally coordinated sites with a distinct local distortion. Typical examples are found in chain silicates.

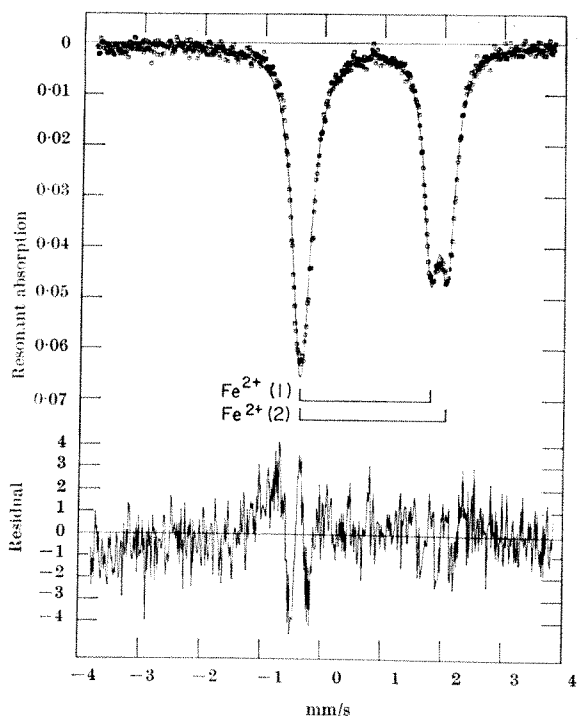


Fig. 1. Nuclear quadrupole split doublets A_1 , B_1 and A_2 , B_2 of ^{57}Fe at the two nonequivalent octahedral positions in olivine $\text{Fe}_{24}\text{Fa}_{74}$ at 315°C . The solid line is a least-squares fit assuming 3 Lorentzians. The peak at -0.4 mm/s is a superimposition of two strongly overlapping peaks, compare "residual" (deviation of the fitted curve from the data). The area ratio of the two peaks B_1 and B_2 is indicative of an almost completely disordered Fe^{2+} distribution.

For example, in crystal structures of the orthopyroxene system $(\text{Mg},\text{Fe})\text{SiO}_3$, ferrous ions show preference for the distorted, octahedrally coordinated M2 position, whereas the magnesium ions occur predominantly at the octahedrally coordinated M1 position which exhibits a more regular local symmetry. An analogous situation exists in the cumingtonite-grunerite system $(\text{Mg},\text{Fe})_7\text{Si}_4\text{O}_{22}(\text{OH})_2$ where iron is predominantly located at the octahedrally coordinated, strongly distorted M4 position, whereas magnesium prefers the less distorted M1, M2 and M3 octahedral positions. Olivines of the composition $(\text{Mg},\text{Fe})\text{SiO}_4$ should therefore show similar differential cation distributions over the octahedrally coordinated sites. But the difference in the distortion between the two nonequivalent octahedral sites M1 and M2 is less than that between M1 and M2 in pyroxenes or M1, M2, M3 and M4 in amphiboles. Thus ordering should be less pronounced compared with the aforementioned chain silicates. In fact, recent structural refinements of four natural olivines close to the $\text{Mg}_2\text{SiO}_4\text{-Fe}_2\text{SiO}_4$ join by Birle *et al.*¹ did not reveal cation ordering within the experimental error.

Some recent preliminary investigations of the cation distribution in olivines using Mössbauer spectroscopy and electronic absorption techniques have led to ambiguous results. Malysheva *et al.*² recently investigated Mössbauer resonant absorption spectra of ^{57}Fe at 550 K in two olivines of the compositions $\text{Fa}_{50}\text{Fo}_{50}$ and $\text{Fa}_9\text{Fo}_{91}$. They found that the iron-rich specimen exhibited a statistical distribution of Fe^{2+} and Mg over M1 and M2 (an experimental error was not reported) whereas the iron-poor olivine showed a small, apparent increase in the population of iron at one of the nonequivalent sites. Grum-Grzhimailo *et al.*³ concluded from electronic absorption studies that Fe^{2+} in a magnesium-rich olivine $\text{Fo}_{92}\text{Fa}_8$ is located at the less distorted M1 sites. They did not exclude the possibility that iron may be present in both positions. Burns⁴ reported a "slight relative enrichment

of Fe^{2+} ions in M2 positions of most Mg-Fe olivines, although the proportion of iron in this site does not exceed 55 per cent of the total iron". In the same article he quoted an accuracy of about 10 per cent so that his statement cannot be considered significant. It is clear from this brief review that precise measurements are essential to determine small cation enrichments at one of the M positions.

The Mössbauer resonant absorption spectra of ^{57}Fe in three olivines were investigated at absorber temperatures between 77 K and 700°C . The specimens were a synthetic fayalite Fe_2SiO_4 , a ferrophonolite $\text{Fo}_{26}\text{Fa}_{74}$, and a chrysolite $\text{Fo}_{92}\text{Fa}_{18}$. The absorber density was approximately 6 mg natural iron/cm². The samples were heated in a regulated platinum furnace at a pressure of less than 10^{-5} mm Hg. No signs of iron oxidation could be detected in the samples after the heat treatment. A typical olivine spectrum is shown in Fig. 1. The spectral results are presented in Tables 1 and 2.

Table 1. NUCLEAR HYPERFINE DOUBLETS OF ^{57}Fe IN OLIVINES: QUADRUPLER SPLITTINGS AND ISOMER SHIFTS

Olivine	Chemical composition	Absorber temperature ($^\circ\text{C}$)	Quadrupole* splitting (mm/s)		Isomer shift† (mm/s)	
			1	2	1	2
Ho-A	$\text{Fo}_{26}\text{Fa}_{74}$	315	2.41	2.14	1.03	0.89
Ho-A	$\text{Fo}_{26}\text{Fa}_{74}$	570	1.95	1.67	0.86	0.72
B1	$\text{Fo}_{26}\text{Fa}_{74}$	315	2.47	2.25	1.01	0.90
B1	$\text{Fo}_{92}\text{Fa}_{18}$	555	2.01	1.77	0.85	0.73
B1	$\text{Fo}_{92}\text{Fa}_{18}$	650	1.85	1.62	0.78	0.67

*Determined from three-line fits to A_1 , B_1 , B_2 (10 variables); the separation between A_1 and A_2 is $\sim 0.20\text{ mm/s}$ for fayalite.

†Referred to metallic iron at room temperature.

Table 2. NUCLEAR HYPERFINE DOUBLETS OF ^{57}Fe IN OLIVINES: LINE WIDTHS AND AREA RATIOS

Olivine	Chemical composition	Absorber temperature ($^\circ\text{C}$)	Line widths (FWHM) (mm/s)			Area ratios†	
			A^*	B_2	B_1	B_1+B_2	B_1
Synthetic	Fa_{100}	320	(0.389)	0.282	0.290	0.507	0.515
Synthetic	Fa_{100}	530	(0.385)	0.288	0.283	0.505	0.497
Ho-A	$\text{Fo}_{26}\text{Fa}_{74}$	315	(0.364)	0.297	0.286	0.485	0.489
Ho-A	$\text{Fo}_{26}\text{Fa}_{74}$	570	(0.361)	0.291	0.302	0.485	0.485
B1	$\text{Fo}_{26}\text{Fa}_{74}$	315	(0.330)	0.313	0.276	0.495	0.444
B1	$\text{Fo}_{92}\text{Fa}_{18}$	555	(0.338)	0.321	0.288	0.495	0.461
B1	$\text{Fo}_{92}\text{Fa}_{18}$	650	(0.344)	0.325	0.301	0.493	0.456
B1 (915 $^\circ\text{C}$)	$\text{Fo}_{92}\text{Fa}_{18}$	360	(0.326)	0.309	0.282	0.498	0.445
B1 (915 $^\circ\text{C}$)	$\text{Fo}_{92}\text{Fa}_{18}$	390	(0.322)	0.298	0.273	0.492	0.456
B1 (915 $^\circ\text{C}$)	$\text{Fo}_{92}\text{Fa}_{18}$	510	(0.332)	0.311	0.294	0.494	0.470

* Apparent width determined from three-line fits (10 variables).

† Determined from four-line fits (13 variables) in the case of fayalite and from three-line fits (10 variables) in case of Ho-A and B1; in the latter cases, four-line fits did not give significant differences.

The spectra consist of two superimposed doublets caused by Fe^{2+} at the M1 and M2 sites. They were generally fitted with four Lorentzians, A_1 , A_2 , B_2 , B_1 (compare Fig. 1) using the least-squares technique (thirteen variables, including one variable for the off-resonance count rate). Each of the two doublets seems to be symmetric in intensity, at least in fayalite. The sums of the areas of the low velocity peaks (A_1 , A_2) and that of the high velocity peaks (B_1 , B_2) are almost identical (see col. 7 in Table 2). Only the two high velocity peaks are sufficiently resolved to enable precise determinations of the peak areas from least-square fits using three variables per Lorentzian. Thus conclusions on the Fe^{2+} distribution can only be deduced from the B_1 and B_2 peaks. Spectra of the same absorber at various temperatures reveal that the area ratio $B_1/(B_1+B_2)$ as well as the total area ratio $(B_1+B_2)/(A_1+A_2+B_2+B_1)$ are nearly independent of the temperature (Table 1). The $B_1/(B_1+B_2)$ ratio in the synthetic fayalite was found to be close to 1/2. This is indicative of an almost identical recoil-free fraction for ^{57}Fe at the M1 and M2 sites ($f_{M1}/f_{M2} = 1.00 \pm 0.02$) in the temperature range of 300 to 500°C . Nothing is known about the variance of f_{M1}/f_{M2} in the $\text{Fe}_2\text{SiO}_4\text{-Mg}_2\text{SiO}_4$ solid solution series.

In column 8 of Table 2 the area ratios of the partially resolved high velocity peaks are presented. The standard

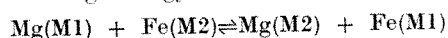
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error determined from replicate spectra is approximately 3–4 per cent. The ratio should be interpreted with precaution because of the strong overlap. The Lorentzian fits to the high velocity peaks were found to be statistically nearly acceptable (compare Fig. 1), but very slight deviations of the peaks from Lorentzian shape may contribute to a significant systematic error. Apparent deviations may be due to a temperature gradient in the absorber or to a very slight oxidation of ferrous iron.

The assignment of the doublets 1 and 2 to the M1 and M2 sites is an unresolved problem. Quadruple splittings in silicates have previously been related to the distortion of the octahedral sites, but the differential splittings and distortions in olivines are too similar for such an argument.

It is concluded that the magnesium and iron distribution in the iron-rich olivine is almost entirely disordered, while in the magnesium-rich sample there seems to be approximately 10 per cent more iron in one of the nonequivalent M positions. Heat treatment of this specimen at 915° C for 24 h and subsequent quenching in liquid nitrogen did not change the distribution. But to draw definite conclusions on the accurate site occupancy from Mössbauer spectra, the two doublets must be assigned unambiguously and the nonequivalent recoil-free fractions of ^{57}Fe must be determined precisely at both octahedral positions.

The apparently different degree of disorder in two samples is not indicative of different crystallization or cooling histories. In fact, the ferrohortonolite sample is an olivine from the abandoned O'Niell iron mine, Orange City, New York⁵, and the magnesium-rich olivine is from the more rapidly cooled upper margin of a perititic sill, Isle of Skye, Scotland^{6,7}. Alternatively the linear relationship between lattice constants and composition in the system $\text{Mg}_2\text{SiO}_4 = \text{Fe}_2\text{SiO}_4$, for example, ref. 8 does not necessarily imply that the magnesium and iron distribution at each nonequivalent site is close to an ideal one, and somewhat different degrees of disordering in crystals with different $\text{Fe}/(\text{Fe} + \text{Mg})$ ratios may correspond to the same equilibrium temperature. From the generally high degree of Mg,Fe disorder in natural olivines it is concluded that the exchange energy for the reaction



must be quite small. The heating experiment at 915° C suggests that the activation energy for the cationic exchange is large, probably a peculiarity of the crystal structure of olivine. It is certainly larger than that for the Mg,Fe exchange in orthopyroxenes⁹.

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Inactivation of the Scrapie Agent by Near Monochromatic Ultraviolet Light

SCRAPIE is a progressive degenerative disease of the central nervous system of sheep. Because the disease is transmitted by cell-free filtrates the agent has been classified as a virus, but its response to many chemical and physical treatments has long been known to differ from that of "conventional" viruses. The transmitting agent increases greatly in quantity in the animal host. In the terminal stages of the disease in mice, preparations from the brain must usually be diluted by a factor of 10^7 – 10^8 to give an average of one mean lethal dose per unit volume, whatever the original inoculum to the affected mouse has been. Haig and Clarke¹, who used a substantial starting inoculum to follow the "growth" of the agent, showed an increase by a factor of 10^4 in the titre of the agent in mouse brain by the time the terminal stage was reached. But experiments with ionizing and ultraviolet radiations led Alper *et al.*^{2,3} to question whether the agent depended on replication of a nucleic acid moiety for proliferation, which was shown to occur also when the titre of the injected material had been reduced by irradiation *in vitro*⁴. The dose of ionizing radiation required to give an average of one inactivating event per infective unit was much larger, and the inferred "target volume" (molecular weight about 1.5×10^5 , ref. 2) therefore much smaller, than for any virus; if the "target" were nucleic acid, this molecular weight would be too low to allow of sufficient coding information for replication. With ultraviolet irradiation at 254 nm, detectable inactivation required doses which were very large compared with those which inactivated even the most "resistant" entities whose function depended on the integrity of nucleic acid^{3,4}. This suggested that the agent might be comparatively transparent to ultraviolet of wavelength in the "germicidal" region in which nucleic acids absorb most strongly.

Inactivation of any biological function by ultraviolet irradiation of a given wavelength requires that the macromolecules on which the function depends absorb energy at that wavelength. If the efficiency with which inactivation occurs is independent of quantum energy, that is, of wavelength, the "action spectrum" resembles the absorption spectrum for the chromophore of the biological macromolecule responsible for the function being tested. (The chromophore is not necessarily itself the region in which the lesion occurs, for energy may be transferred between parts of a macromolecule.)

Because the doses used at different ultraviolet wavelengths were too small to induce measurable inactivation of the scrapie agent, an earlier attempt to measure an action spectrum failed⁵. Subsequently, when larger doses were used at 254 nm, from a germicidal lamp, and at 280 nm, from a powerful monochromator⁶, these two wavelengths proved to be equally effective (unpublished results of D. A. H., M. C. C., T. A. and W. A. Cramp). But it was not practicable to examine effects of ultraviolet irradiation at shorter wavelengths because the output of the monochromator was too low in the region below 250 nm.

The development at the Institut du Radium of a system for obtaining high fluences of near monochromatic light has now enabled us to compare the effectiveness of bands centred at 237, 250 and 280 nm, using doses large enough to give considerably more inactivation of the agent than had previously been possible (ref. 3 and unpublished results of D. A. H., M. C. C., T. A. and W. A. Cramp). Light at 254 nm from a germicidal lamp was also used. The results are sufficiently clear cut and informative to warrant a preliminary communication.

The radiation system used has been described by Muel and Malpéce⁶. Light from a 500 W high pressure mercury lamp was passed through appropriate liquid filters so arranged that a horizontal beam of near monochromatic light fell on the irradiation vessel, which was 10 cm² in section and 2 mm deep. The samples were stirred continuously during irradiation, and the temperature was kept at 2° C. The dose was monitored during irradiation. Chemical dosimetry⁷ was used to determine the incident dose received by the samples, except in the case of the germicidal lamp, with which physical measurements were made. Corrections were applied to obtain average doses, these being based on measurements of the optical density of the material at the relevant wavelength. The spectral distributions for the three filter systems used are shown in Fig. 1. The half width of the bands was about 15 nm.

The methods for estimating the activity of the scrapie samples were as previously described^{2,3}, except that the mice were observed for a total of 40 weeks, to ensure that the irradiation was not merely delaying the onset of symptoms. Results were the same, whether observations made at 32 or 40 weeks were used to compute residual activity. Values of LD₅₀ and their ratios to that for the control were determined using a computer program incorporating probit analysis⁸.

Table 1. RAW DATA FOR INACTIVATION OF SCRAPIE AGENT BY ULTRAVIOLET IRRADIATION AT VARIOUS WAVELENGTHS

Wavelength peak (nm)	254		250		280		237		Control*
Dose ($\times 10^4$ ergs/mm ²)	4.3	14.3	3.7	6.5	4.3	10.0	1.3	2.9	(not irradiated)
Dilution of sample (log ₁₀)	Cases of scrapie/number of mice per group								
3:602	8/8	6/6	7/7	8/8	8/8	8/8	7/7	3/8	23/23
4:602	8/8	3/7	8/8	8/8	8/8	8/8	7/7	1/8	20/20
5:602	7/8	1/8	8/8	5/7	7/8	2/7	4/8	0/6	23/23
6:602	2/6	0/7	5/8	0/8	4/6	0/8	2/7	0/8	22/23
7:602	1/8	0/8	1/7	0/8	0/7	0/7	0/8	0/8	12/20
8:602	0/7	0/8	0/7	0/8	0/8	0/8	0/8	0/8	1/24
Dilution for LD ₅₀ (probit analysis)									
log ₁₀	6.64	4.62	6.88	5.80	6.57	5.40	5.89	3.56	7.69
95 per cent confidence interval	to 6.30	to 4.29	to 6.56	to 5.47	to 6.23	to 5.08	to 5.57	to 3.18	to 7.49
	6.97	4.95	7.20	6.13	6.92	5.73	6.21	3.91	7.88
Residual activity, fraction of control log ₁₀	2.95	4.94	1.19	2.11	2.80	3.72	2.20	5.87	
95 per cent confidence interval	to 2.56	to 4.55	to 2.81	to 3.73	to 2.49	to 3.34	to 3.83	to 5.45	
	1.34	3.32	1.57	2.49	1.28	2.10	2.58	4.28	

* Results for three independent control samples pooled.

The raw data for irradiation at 237, 250, 254 and 280 nm are presented in Table 1, and the inactivation curves in Fig. 2. It is important that (a) inactivation curves were

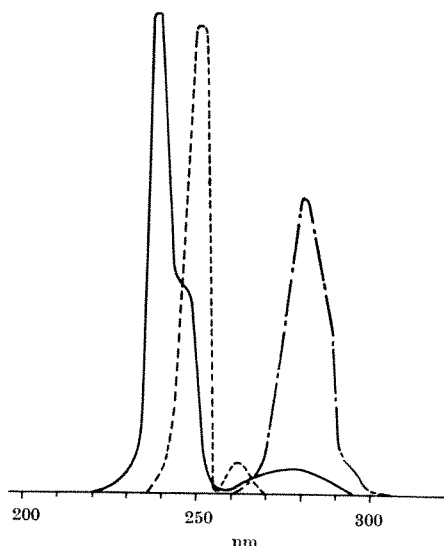


Fig. 1. Spectral distribution of photon fluxes for the three sets of filter combinations used. Ordinate scale arbitrary, different for each set of filters.

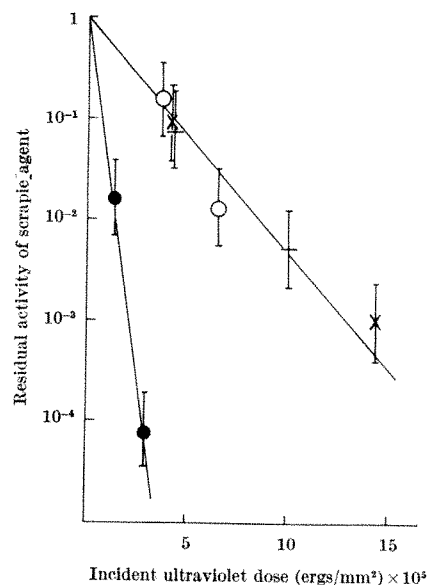


Fig. 2. Inactivation of scrapie agent by ultraviolet irradiation at different wavelength peaks. ●, 237 nm; ○, 250 nm; ×, 254 nm; +, 280 nm. Vertical bars show 95 per cent confidence intervals for ratios to controls.

exponential; (b) the efficiency of inactivation at 250 and 280 nm was the same, within experimental error, in confirmation of unpublished results obtained previously, and (c) inactivation by the waveband with peak at 237 nm was about six times as effective as that by the higher wavelengths.

The effectiveness of radiation at 237 and 280 nm, expressed relative to that at 250 nm, is shown in Fig. 3.

The shaded area of Fig. 3 shows the region within which action spectra lie for many systems (refs. 9-16 and unpublished results of B. M.) which depend on nucleic acid for their function (for example, infectivity of viruses, transforming ability of DNA, integrity of genetic characters). These spectra have the same characteristics as the absorption spectra of nucleic acids: in general, a maximum "cross-section" for ultraviolet wavelengths of 250 to 270 nm, and, with viruses, a minimum in the range 235-245 nm. The difference in the response of the scrapie agent is striking. Table 2 gives some comparative data for certain absorption spectra and for inactivation of the protein-synthesizing function of ribosomes of *Escherichia coli*¹⁸, determined by the same irradiation techniques as were used with the scrapie agent. Data for irradiation of complete tobacco mosaic virus (TMV)¹⁹ of the U1 strain are also shown: the action spectrum for this virus is exceptional in that it does not conform with the usual pattern (Fig. 3), although that for the free ribonucleic acid of TMV corresponds precisely with its absorption spectrum⁹.

There is need for caution in the interpretation of action spectra, for energy transfer processes, or interactions between parts of a complex, may affect the end result.

Table 2. SOME DATA FOR COMPARATIVE ABSORPTION AND EFFECTIVENESS AT THREE WAVELENGTHS

Test system	Absorbance at wavelength (nm)			Refs.
	237	250	280	
RNA of potato virus X	0.48	1	0.33	16
Bovine serum albumin	2.5	1	1.3	17
Hyaluronic acid	1.91	1	1.17	17
Purified bacterial endotoxin (lipopolysaccharide-protein complex)	2.0	1	0.8	18
Effectiveness for inactivation				
Transforming DNA; free RNA of TMV; TMV strain U2				
Various RNA and DNA viruses (Fig. 3)	0.4-0.7	1	0.5-0.8	9-16*
Ribosomes of <i>E. coli</i>	1.45	1	0.5	18
Tobacco mosaic virus strain U1 (whole)	2.3	1	0.39	19
Scrapie agent	6	1	1	

* And unpublished results of B. M.

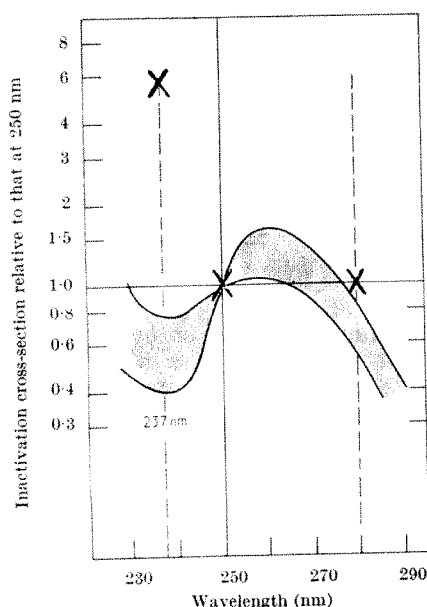


Fig. 3. X, Effectiveness of ultraviolet at 237 and 280 nm (peak) relative to that at 250 nm for inactivating the scrapie agent. Shaded area marks region in which many action spectra lie for biologically active DNA and for viruses (refs. 9-16 and unpublished results of B. M.).

This seems to be the case with the U1 (though not the U2) strain of TMV, in which the whole virus is strongly protected in the wavelength region which most effectively inactivates the free RNA, a phenomenon still not satisfactorily explained²⁰. With this reservation in mind, we think it justifiable to postulate that the chromophore responsible for ultraviolet inactivation of the scrapie agent could be other than a part of a nucleic acid molecule or of a nucleoprotein complex.

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Superfoetation of Mice

DURING experiments in our laboratory on the effects of environmental temperature on mice, anomalous gestations have been observed. We give here examples of the birth of litters too soon after a previous litter to be the result of a normal post-partum insemination. In this account, by "superfoetation" we mean the occurrence in the same animal of two pregnancies at different stages of development¹.

The mice were of a mixed stock derived from four inbred strains, A, A2G, C57Bl and GFF. They are very fertile². There were two breeding colonies, one in a room kept at about 21° C, the other at -3° C. The members of pairs were always the young of different parents. They were permanently mated at the stage of 5 to 8 weeks. First litters were weaned at 3 weeks. Second litters were killed at birth. After the birth of the second litter, females were examined twice daily, at 0900 and 1630 h, for the presence of a vaginal plug. A plug was taken to indicate day 1 of pregnancy. Females were killed and dissected on day 16 of this, the third gestation. The structural age of the foetuses was recorded on the basis of the scheme proposed by Grüneberg³.

The modal structural age was 16 days at 21° C, fifteen days at -3° C. Nevertheless, in both environments some litters were born before the supposed sixteenth day of gestation. When this first happened a recording error was suspected; but repetition made this unlikely. Moreover, the anomalies were confined to the hybrid stock: mice of two inbred strains treated in the same way reproduced normally.

Accordingly, more rigorously timed third pregnancies were arranged. Hybrid mice in both temperatures were treated as before until just before the birth of the second litter. The male was then removed. The second litter was killed. On the fifth day after parturition the male was replaced at 1700 h, and was left with the female for 16 h. The females were again killed and dissected on the presumed day 16 of pregnancy, and the modal structural ages of the foetuses were as before; but, of sixty mice so mated, six, listed in Table 1, gave birth to normal litters before day 16.

Table 1. PREMATURE THIRD LITTERS

Mouse No.	First litter No. born	First litter No. weaned	Second litter No. born	First interval, days	Third litter No. born	Third litter No. weaned	Days since last mating
4	9	7	12	21	10	10	14
6	3	3	6	21	4	4	13
9	12	0	9	21	14	12	16
24	14	0	13	21	10	7	16
30	2	0	7	20	11	11	14
40	7	0	14	20	13	10	16

The last four were bred at -3° C

Table 2. "FATHERLESS" THIRD LITTERS

Mouse No.	First litter No. born	First litter No. weaned	Second litter No. born	First interval, days	Third litter No. born	Third litter No. weaned	Second interval, days
N3	6	6	7	25	10	8	21
N4	1	0	4	20	2	0	18
N6	9	0	9	19	15	14	19

It was hypothesized that these litters were the result of inseminations that had taken place before the births of litter 2. Accordingly, a further twenty-two pairs were mated, all at 21° C, and treated in the same manner as the sixty of the first group, except that the male was not replaced at any time after the birth of the second litter. Nevertheless, three of the mice gave birth to third litters (Table 2). Both sexes were represented in these litters, and members of two of them were mated and proved to be fertile.

Superfoetation of mice can be induced experimentally, by means of hormone treatment and artificial insemination⁴, but this has little bearing on our findings. Although reports of apparent "natural" superfoetation of mice have been published, personal enquiry has shown that they are not well known, even to workers on mouse reproduction. Perhaps they have been disregarded because unrepeatable. Crew and Mirskaia, in a brief note⁵, listed six examples, out of 100 mice closely observed, of anomalous births which they attributed to insemination during the previous pregnancy. Watt, on the basis of a single observation, concluded⁶ that oestrus and coitus could occur during pregnancy. Littleford and Gysin give⁷ five examples of premature litters, of which they consider two "to admit of no other explanation" than superfoetation. They also review the literature up to 1944, including that on other species.

If it is accepted that mice can sometimes produce litters at intervals of 16 days or less, there are, in principle, several possible explanations. We exclude parthenogenesis, for the products of this process should only be females. The hypothesis of a "pre-partum" oestrus, and insemination during late pregnancy, faces the difficulty that the spermatozoa would have an excessively difficult journey up the horns of the pregnant uterus. There is a theoretical possibility that sperm could remain from a previous insemination, and could fertilize ova released at a pre-partum oestrus; but the spermatozoa of mammals are usually described as having an effective life of only a few hours^{8,9}. A final hypothesis is of retention in the Fallopian tubes of unimplanted embryos during pregnancy. If there were no further delay of implantation, these embryos could become implanted immediately after the birth of the normally developing litter, and could then be born about 15 days later. There is already evidence of the ability of mouse embryos to survive unimplanted for much longer than the normal period of 4 days¹⁰. Rollhäuser has reported¹¹ a single example of apparent oestrus and effective insemination early in pregnancy. Such anomalous matings could have occurred among our mice, and have produced blastocysts the implantation of which was delayed until birth of the litter already developing.

Deanesley, in a recent review¹, describes superfoetation in mammals generally as "certainly very rare". Our experiments are evidently the first examples of superfoetation of mice deliberately engendered without hormonal interference. If they can be repeated with sufficient regularity, they suggest interesting possibilities for the study of foetal and maternal physiology.

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Effect of Anti-luteinizing Hormone Serum on the Ovulation of Rats

In the cyclic female albino rat, a release of pituitary luteinizing hormone (LH) occurs on the afternoon of proestrus¹⁻³. This apparently induces ovulation, for ova are seen in the Fallopian tube 12 h later. Similarly, it is well known that in immature rats primed with pregnant mare serum gonadotrophin (PMS), ovulation can be induced by the administration of human chorionic gonadotrophin (HCG) or LH, the ova being seen in the Fallopian tube 12 h later. No information is available, however, about the mode of action of LH, released or administered, in bringing about ovulation. We have approached this problem by blocking the action of the ovulating hormone (LH) at various times after administration.

As blocking agent we used a specific and well characterized rabbit antiserum to highly purified LH. The details of immunization schedule and characterization of the antiserum have been described before^{6,7}. The antibody titre of the antiserum was determined by the quantitative precipitin technique⁸, and the amount of antibody administered was generally slightly more than necessary completely to neutralize the LH administered⁷.

Immature 25-day-old female rats of our institute colony maintained on a regime of 14 h light and 10 h dark (light from 0600 h to 2000 h) were primed with two subcutaneous injections of 50 μ r of PMS 48 h apart. An ovulating dose of 100 μ g of ovine LH was administered by the intracardiac route 56 h after the second injection of PMS. Circulating LH was neutralized with 0.2 ml. of rabbit anti-ovine LH serum, also given by the intracardiac route. Different groups of rats received the antiserum at different times after the ovulating dose of LH, so that the LH acted for various periods. The control group received normal rabbit serum (NRS) by the intracardiac route 1 h after injection of LH. The rats were autopsied 18 to 22 h after injection of hormone and the Fallopian tubes were examined for ova as described by Zarrow *et al.*⁹. Table 1 shows that the minimum time necessary for LH to cause ovulation (minimum effective time—MET) is between 1 and 2 h.

Table 1. EFFECT OF NEUTRALIZATION OF LH AT VARIOUS TIMES ON SUPER-OVULATION

Time of antiserum injection after LH treatment	Rats ovulated/total	Average No. of ova/total
NRS control 1 h	8/8	22.4/179
Antiserum 10 min	0/4	0/0
" 30 min	0/7	0/0
" 1 h	1/7	1/7
" 2 h	7/9	8/71
" 6 h	6/6	14.5/87

Hormone (100 μ g of LH) and the serum sample (0.2 ml.) were injected by the intracardiac route.

The half-life of LH in circulation is only about 25 min^{10,11} and so it seems that LH is retained in some form or other for much longer in the ovary, thereby extending the "functional life" of this hormone. It is interesting that Eshkol and Lunenfeld¹², using labelled HCG in mice, found that although the concentration of hormone in circulation had declined to a base level after 90 min, it was retained by the ovary for up to 4 h. It may be interesting to point out that the critical period of 2 h observed on the proestrous afternoon in the female rat (when endogenous LH is supposed to be released²) coincides with the MET of approximately 2 h that we observed.

Sasamoto¹³, after using HCG antiserum in mice treated with PMS-HCG, concluded that a minimum of 2 h of circulation of HCG was necessary for 100 per cent ovulation. He, however, obtained between 25–50 per cent ovulation even when HCG was neutralized at intervals of 30 and 60 min.

The use of metabolic inhibitors, such as actinomycin D, puromycin and cycloheximide^{14–16}, has shown that in

rabbits, hamsters and frogs there is a similar 2-4 h dependence on pituitary gonadotrophic hormone, during which the ovulating hormone seems to initiate ovulation. Barros and Austin¹⁵, however, observed that ovulation in the hamster could be inhibited by actinomycin D during the first 5.5 h after injection of HCG, and that the percentage of inhibition decreased with time.

For ovulation to occur, the mature ovarian follicles of these animals seem to need pituitary trophic stimulus only for a minimum time, after which the follicles become autonomous and complete ovulation on their own. It is interesting that Pool and Lipner¹⁴ suggested that this initial stimulation of ovulation by LH involved the synthesis of new protein and RNA.

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Evidence for a Male Sex Pheromone in the Noctuid, *Trichoplusia ni*

THE hairpencils of several male Lepidoptera secrete volatile substances¹⁻³. Aplin and Birch¹ reported that the scent-brushes of some noctuids contain benzaldehyde as their chief component and they suggested that this substance might function as an aphrodisiac because the brushes were displayed during courtship, but they gave no evidence that the compound was olfactorily or otherwise stimulating to the insect. Recent electrophysiological experiments⁴, however, have demonstrated that the hairpencil substances of the male queen butterfly are detected by the female antenna and behavioural studies^{5,6} have shown them to function as a female aphrodisiac. This report provides evidence that the hairpencils of the male cabbage looper, *Trichoplusia ni*, produce a volatile secretion which probably stimulates the female during courtship.

In laboratory cages, the male looper approaches a calling female from the rear and waves his antennae over

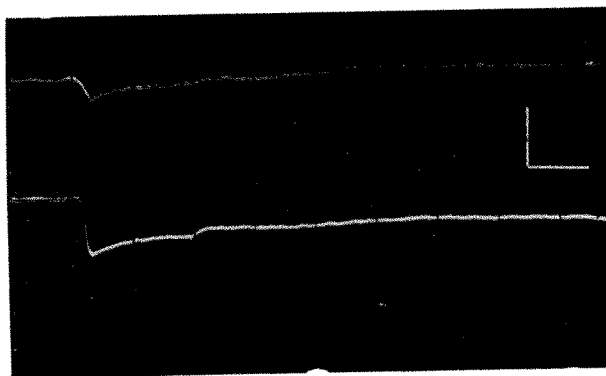


Fig. 1. Electroantennograms from female cabbage looper antenna. Upper trace is the EAG response to the control air stimulus; lower trace is the EAG response to the excised hairpencils from a male cabbage looper. Stimulus duration was 1 s; vertical scale, 1.0 mV; horizontal scale, 0.5 s.

her abdomen. He then moves to one side of the female and by a lateral thrust of his abdomen brings his genitalia towards hers causing the external tufts of brown hair that normally lie flat over the last few male abdominal segments to fan out⁷. The genitalia are then extruded and if extended far enough two internal scent-brushes appear and spread briefly. There is no attempt to brush the female antennae with these hairpencils, an integral part of the courtship pattern in the queen butterfly⁵. These observations suggest that the hairpencil substances must act on olfactory rather than contact chemoreceptors on the antennae during courtship¹⁰.

The hairpencils consist of two tufts of 4 mm long hollow scales that arise from membranous tubes on the ventral surface of the eighth abdominal segment. Two areas of glandular tissue are associated with these scales. At the base of the scent-brush itself are large flask-shaped cells that are bound into lobes by nonglandular interstitial cells and resemble a similar gland in *Vitula edmandsae*¹¹. The gland cells have a long narrow reservoir lined with microvilli that communicates with the hollow scent scale. While fully 150-200 μ m long in the newly emerged male, the cells are completely atrophied within two days. The other glandular cells, found on the eighth abdominal sternite, possess minute fan-shaped scales that line the dorsal wall of the fold containing the hairpencils. These cells do not atrophy but remain continuously active.

Hairpencil scales for electrophysiological tests were excised above the gland and inserted into a glass cartridge which was then connected to a hose delivering air at 2.8 l./min. An air pulse of 1 s duration was allowed to pass through the cartridge containing the hairpencils and then over the female antenna. The response recorded from the preparation was the electroantennogram (EAG) obtained by means of Ag/AgCl electrodes^{6,12}. The hairpencil stimulus elicited a slow monophasic negative EAG of 0.8 to 1.0 mV amplitude (Fig. 1) as did methylene chloride extracts containing one male equivalent. In the case of the queen butterfly, excised hairpencils were as effective as the pure synthesized pheromone⁶. On the other hand, the external tufts of brown hair on the looper abdomen failed to elicit an EAG greater than the control.

It is of interest that the male looper, like the male queen butterfly, also responds to its own hairpencil substances and with the same EAG amplitude-response characteristics as the female. By contrast, no female moth^{6,10}, including a noctuid¹³, has been shown to detect her own pheromone, but the female cabbage looper seems to be an exception. A 1 μ l. dose of synthesized female pheromone (*cis*-7-dodecen-1-ol acetate) on filter paper elicits an EAG of about 1 mV from the female while the male response is 3 mV or more. These responses to the male and female pheromones suggest that the antennae of both sexes use similar sensilla for detecting the male

pheromone but possess or use different sensilla for the female pheromone. This conclusion is supported by observations¹⁴ of sexual dimorphism in the antenna of the noctuid, *Heliothis zea*, whose chemoreceptors very closely resemble those of the cabbage looper.

These results and those of Aplin and Birch¹ suggest that the hairpencils of noctuid moths contain compounds which probably function as a female aphrodisiac. It cannot be ruled out, however, that they might also provide an important stimulus to the male during courtship. The difficulty in pinpointing the true hairpencil function in nocturnal moths is the apparent lack of an overt behavioural response in the female (or male) as is found in danaid butterflies⁵, and the difficulty in the laboratory of observing such behaviour in the necessarily dim illumination.

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Evolutionary Significance of Virus Infection

VIRUS infection is widespread throughout the animal and plant kingdoms, and produces most human illness¹, possibly including certain types of cancer². Particles with viral morphology are easily isolated from sea³ or river water suggesting that they are ubiquitous. In this communication we have assembled evidence to support the view that viral transduction is a key mechanism for transporting segments of DNA across species and phylum barriers, and that evolution depends largely on this transfer. The evidence may be summarized as follows.

First, if virus infection served no useful function, evolution of effective means for its elimination might be expected. "Natural" resistance is often due to lack of specific virus attachment sites⁴. One may ask why these persist if their sole function is to assist in acquiring illness. Interferon gives protection⁵ demonstrating that protection is possible. But it is made normally only after infection and is transient⁶. Similarly, humoral and cellular immunity, though apparently under genetic control⁷, appear late in infection. We conclude that total prevention of infection should be possible: the fact that it does not occur suggests that susceptibility to infection confers some advantages on the infected organism.

Second, many viruses cross species barriers with ease,

and are often transmitted in nature directly from members of one phylum to another. If the extent of this crossing were known and fully mapped, pathways of infection would probably be found which interconnect each cell, through other cells, to all other cells—both plant and animal. Although some viruses exhibit surprising cell specificity, others, as, for example, arboviruses, are naturally transmitted from insects to vertebrates and back¹.

Third, incorporation of segments of host DNA into infective virions and subsequent transfer to other cells is well known⁸. The transferred DNA has been shown to be incorporated into chromosomal DNA of bacterial cells^{8,9}. In animal cells, DNA from two different viruses has been found incorporated in the same virion¹⁰, while host DNA has been shown to be incorporated in polyoma¹¹ and SV40¹² capsids. As Trilling and Axelrod noted, the presence of SV40 pseudovirions containing sufficient host nucleic acid for the coding of four or five host proteins suggests an efficient mechanism for gene transfer from one cell to another¹². There are often barriers to adoption of larger segments¹³, but these may not affect shorter strands.

Fourth, whole virus genomes may be incorporated into germ cells and transmitted from one generation to the next^{1,14}. Whether DNA, fortuitously attached during a previous sojourn of the virus in another species, is also carried along does not seem to have been settled as far as higher animals are concerned.

The fifth point concerns parallel evolution, as observed repeatedly in different species presented with the same problems of environmental stress. While squid and vertebrate eyes differ in many details, they solve the same basic problems and each would benefit enormously from bits and pieces of plans interchanged. A continuous flow and interchange of gene parts "on approval" would both explain and facilitate parallel evolution.

The sixth argument concerns the universality of the genetic code. A long history of evolutionary changes obviously lies behind it¹⁵. Why is only one version left? If information from the entire biome was read and is to be read by any and all organisms, only one code could (and would) survive.

The seventh and most convincing argument concerns the difficulty of an evolution based on many small changes which are often inconsequential¹⁶, but with choice dictated by survival. The problem is illustrated by this analogy. A parliament governs by passing or rejecting laws. If the laws considered are always the same, changed one word at a time, can this deciding body function effectively? How much more convenient it would be to consider, occasionally, whole laws or sections of them from foreign sources. These new statutes may be in direct conflict with existing order and may confuse administration in a malignant fashion; or, with small alterations, they may improve governance.

The greatest objection to the concepts presented here is that they undermine the foundations of a favourite pastime—the reconstruction of evolutionary relationships by comparing amino-acid sequences¹⁷⁻¹⁹. The differences observed may as well indicate the number of different virus transductions or transeapsidations required to pass from one animal to a distantly related one. The available data concern homologous proteins of well established importance and do not yield much insight on how new proteins may originate which bear little resemblance to old ones. A corollary of those ideas is that plants or animals which are free of virus infection would evolve very slowly if at all.

Stability of external form during long periods of time may merely reflect the fact that a form more suitable cannot be evolved. Internal biochemistry, however, may have evolved and may be continually evolving in all organisms in parallel. Palaeontology may therefore not accurately reflect either the rate or course of much of molecular evolution.

The essence of the idea described here is that the evolution of one organism depends on contributions and new ideas from all.

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Encephalitis in Newborn Hamsters after Intracerebral Injection of Attenuated Human Measles Virus

ACUTE encephalitis following measles infection in humans usually develops within a few days of the exanthema and can be fatal within 24 h. The presence of intracerebral infective measles virus in one such fatal human case may have been demonstrated by Shaffer *et al.*¹ who inoculated a brain emulsion into two macaques and noted development of clinical measles. Animals also develop acute measles encephalitis. Suckling mice, rats and hamsters of varying ages have shown acute symptoms after intracerebral inoculation of neurotropic strains of measles virus²⁻⁴. The mouse model has been carefully studied⁵ using a mouse-adapted strain of measles virus and monitoring the virus by infectious assays and electron microscopy. The onset of symptoms of encephalitis corresponded both to the development of a minimal level of infective virus within the brain and to the presence of observable nucleocapsids within the dying cell. The experimental study described here was undertaken to determine the effect of attenuated measles virus in newborn hamsters after intracerebral inoculation.

The Edmonston strain of measles virus, which has been used in previous studies in this laboratory^{6,7}, was passaged six times in the BSC-1 monkey cell line after isolation in human cells. The Schwarz strain of measles virus was also passed six times in BSC-1 cells. The history of mouse-adapted measles virus has been previously described^{8,9}; it has been passed a total of twenty-

three times by intracerebral inoculation into newborn mice. Brain homogenates were prepared and titrated according to previously published methods^{6,9}. Random bred golden Syrian hamsters and Swiss-Webster mice were used throughout the study.

A 0.02 ml. intracerebral inoculum of the Schwarz attenuated virus with a titre of 5×10^4 plaque-forming units (p.f.u.)/ml. in BSC-1 cells was administered to twelve newborn hamsters within 24 h of birth, and all developed signs of intracerebral viral damage within 7-10 days of inoculation (Table 1). The hamsters became hyperactive, then hypoactive and finally moribund. Moribund hamsters were killed, and a 20 per cent (w/v) brain suspension was made in Hanks balanced salt solution and titred on BSC-1 cell monolayers. The material contained 1×10^4 p.f.u./ml. of suspension. A 0.02 ml. inoculum of this brain homogenate was inoculated into a second group of newborn hamsters. Five days later, all fifteen of the animals surviving the intracerebral inoculation became hyperactive, then hypoactive, and moribund. The brain suspension resulting from this second intracerebral passage of Schwarz strain virus contained more than a hundred times more virus (2.5×10^6 p.f.u./ml.) when compared with the 20 per cent homogenate from the first passage (1.0×10^4 p.f.u./ml.). A third passage through the brain of this attenuated measles virus shortened the incubation period to 4 days.

Table 1. EFFECT OF VARIOUS STRAINS OF MEASLES VIRUS INOCULATED INTRACEREBRALLY IN NEWBORN HAMSTERS

Virus	Input titre (p.f.u./ml.)	Brain titre (p.f.u./ml.)	No. hamsters surviving injection	No. hamsters with signs of encephalitis	Day of onset	Passage No. in hamsters
Schwarz	5.0×10^4	1.0×10^4	12	12	7,7,7,7,8,8,10,10,10,10,10,10	1
Schwarz	1.0×10^4	2.5×10^6	15	15	All day 5	2
Schwarz	2.5×10^4	Not done	4	4	All day 4	3
Edmonston	4.0×10^4	7.5×10^4	30	1	10	1
Edmonston	7.5×10^4	3.5×10^6	16	10	6,6,8,8,9,9,10,10,11,12	2
Mouse-adapted measles	1.0×10^6	2.0×10^4	13	13	All day 5	1

Table 2. EFFECT OF HAMSTER BRAIN ADAPTED MEASLES VIRUS STRAINS INOCULATED INTO NEWBORN MICE

Virus	Input titre (p.f.u./ml.)	Brain titre (p.f.u./ml.)	No. mice surviving injection	No. mice with signs of encephalitis	Day of onset	Passage No. in mouse brain
Schwarz	2.5×10^4	2.0×10^4	11	11	5,6,6,7,7,7,7,7,7,7,7	1
Schwarz	2.0×10^4	Not done	8	8	3,4,4,5,5,6,6,6	2
Edmonston	3.5×10^4	8.7×10^4	8	8	7,7,8,8,8,8,8,8	1
Edmonston	8.7×10^4	Not done	6	6	6,6,6,6,6,6	2
Mouse-adapted measles	2.0×10^4	4.6×10^5	11	11	5,5,5,5,5,6,6,6,8,13,13	24
Schwarz	5.0×10^4	Not done	10	0	—	0
Edmonston	2.0×10^5	Not done	10	0	—	0

The Edmonston strain of measles virus was also transferred directly from BSC-1 cells to newborn hamster brain. Similar signs of cerebral viral damage were seen in only one animal although the virus input was similar to that used with the attenuated virus. Little virus was produced, but its passage into newborn hamsters yielded results comparable with those obtained with the attenuated virus (shortened latent period and increase in intracerebral virus).

The Imagawa strain of Edmonston measles virus which is adapted to mouse brain successfully adapted to hamster brain in the first passage. All the inoculated hamsters showed signs of viral damage in the central nervous system within 5 days of inoculation (Table 1).

Suspensions of hamster brain containing high titres of Edmonston, Schwarz or Imagawa strains of measles virus were inoculated intracerebrally into newborn mice (Table 2). Signs of damage to the central nervous system occurred in all mice injected with the infected hamster brain suspensions, but not in those injected with virus grown in tissue culture. Hyperactivity was most marked in mice receiving the Imagawa strain of virus and less apparent in those receiving the Schwarz strain. Animals inoculated with any of the three brain homogenates, however, eventually became hypoactive and moribund before they were killed.

All three hamster-adapted strains of measles virus replicated in newborn mice following intracerebral inoculation. Titres recovered from mouse brains equalled or exceeded the titres in the inocula. To confirm the identity of the virus causing the observed effects, dilutions of Schwarz and Edmonston mouse brain suspensions were reacted with convalescent human measles serum. The serum completely neutralized the plaque-forming ability of the brain suspension in BSC-1 cells.

The development of a neurotropic strain of measles virus for laboratory animals is not new. Imagawa and Adams² adapted the Edmonston strain to mice, although several passages were necessary to produce consistent symptoms of encephalitis and high titres. Waksman *et al.*⁴ likewise adapted the Philadelphia 26 strain to newborn hamster brain after four passages. The Edmonston strain, however, was found by Feldman (personal communication) to initiate signs of encephalitis in newborn hamsters without several blind brain passages. This led to the growth of non-neural adapted Edmonston virus growing in neonate hamster cerebellar cells *in vitro*⁹. Evidence for the intracerebral propagation of measles virus after intracerebral inoculation of Schwarz measles strain and the subsequent clinical signs of acute measles encephalitis in the neonatal animals in this study occurred soon after the initial introduction of the virus. No blind passages were needed. The resultant titres of infected brain homogenates were higher after inoculation of the attenuated virus and signs related to clinical measles encephalitis occurred in a greater percentage of animals than with the supposedly more virulent Edmonston virus. Thus in the brain of a susceptible animal, the hamster, the Schwarz strain seemed to be more virulent than the parental Edmonston virus.

The Schwarz type of vaccine might also initiate clinically acute measles encephalitis in humans. Pestri¹⁰ described such a case in a 14 month old female infant 11 days after vaccination. Schneck's¹¹ observation that subacute sclerosing panencephalitis (SSPE) developed 3 weeks after live measles vaccination adds support to the concept that attenuated virus may cause damage to the central nervous system. Recently, Payne (personal communication) took a brain homogenate inoculum from a patient with SSPE and was able to produce symptoms of acute encephalitis after inoculation into newborn hamsters. *In vitro* tests showed that this homogenate contained infective virus. Combining the various observations reported so far, the hamster brain seemed to be a promising animal model in which to study the pathophysiology of SSPE and possibly multiple sclerosis. The observations reported also suggest that further attenuated measles virus strains without neurological potential in the newborn hamster should be developed for possible use in the prevention of measles in the human population.

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Growth of Porcine Transmissible Gastroenteritis (TGE) Virus in Organ Cultures of Pig Tissue

It has been shown recently that it is possible to grow viruses in organ cultures of human (ref. 1 and personal communication from D. Egglestone) and mouse¹ intestinal tract. It seemed to us that these cultures might support the growth of viruses of the alimentary tract of man which are postulated to cause diseases such as acute gastroenteritis and winter vomiting disease. Transmissible gastroenteritis (TGE) of pigs resembles these diseases in many ways², and the virus can be detected and precisely titrated in tissue cultures^{3,4}; it therefore seemed to be a suitable model on which to test the hypothesis that a gastroenteritis virus might grow in organ cultures of the gut.

Table 1. RESULTS OF GROWTH CURVE EXPERIMENTS OF TGE VIRUS IN PIG INTESTINAL ORGAN CULTURES

Tissue	Culture	Day 0	P.F.U./tube or dish in harvests				
			Day 1	Day 2	Day 3	Day 4	Day 6
Oesophagus	AA	0	30	206	130	282	7
Oesophagus	RT	0	3	47	393	260	240
Stomach	AA	0	0	0	0	0	0
Stomach	RT	0	0	0	0	0	0
Jejunum	AA	0	0	0	0	0	0
Jejunum	RT	0	0	0	0	0	0
Ileum	AA	0	7	257	550	265	0
Ileum	RT	0	7	187	80	427	27
Caecum	AA	—	75	250	182	100	65
Caecum	RT	—	47	107	170	113	40
Colon	AA	—	50	43	0	0	7
Colon	RT	—	193	347	30	3	10
Nasal epithelium	AA	—	0	0	10	40	7
Nasal epithelium	RT	—	0	0	0	0	0

TGE inoculum 1.7×10^3 P.F.U./tube or dish.

AA = plastic Petri dish culture; RT = roller tube culture.

The virus used was strain FS 216/64 passaged many times in pig kidney cells, and subsequently in secondary adult pig thyroid (APT) tissue cultures. It was titrated as plaque-forming units in monolayer cultures of APT cells⁴. Organ cultures were prepared from the tissues of piglets obtained at the 112th day of gestation by caesarean section, delivered into a germ-free plastic isolator and killed by injection of sodium pentobarbitone. The cultures were set up in plastic Petri dishes or in roller tube cultures as previously described¹. The medium was Eagle's medium with 0.2 per cent bovine plasma albumin. The cultures were maintained at 37° C, the dish cultures in a humidified CO₂ incubator.

Cultures were set up and inoculated with 0.1 ml. of virus pool. After 3 h of incubation they were washed three times. The medium was removed each day, mixed with an equal volume of broth and then stored at -70° C until titrated. The results of an experiment are shown in Table 1. After washing there was no trace of the virus inoculated, and its subsequent appearance in cultures

of oesophagus, ileum, caecum and colon strongly suggests that it was multiplying in these tissues. It can be seen that 550 p.f.u. were recovered from cultures of ileum which were washed three times and changed four times, so that the original inoculum of 1,700 p.f.u. had been diluted by an estimated factor of 10^{-7} . It apparently grew as well in roller tube cultures as it did in dish cultures, but did not grow at all in cultures of trachea, although small amounts of virus were present in later harvests from one culture of nasal epithelium.

Six days after preparation the cultures were fixed, sectioned and stained. There was well preserved epithelium in cultures of nasal, tracheal, colonic and caecal mucosa. Most of the highly differentiated epithelium of stomach and small intestine was shed or necrotic. Dedifferentiated cuboidal or flattened epithelium was seen, however, particularly at the edges of the organ cultures. The cells were better preserved in dish cultures than in the tube cultures, and nothing was seen which suggested a specific viral lesion.

It was concluded that the TGE virus grows in organ cultures. We do not understand why it failed to grow in stomach, but this tissue supports the growth of a number of other viruses very poorly¹. The failure to grow in respiratory epithelium was expected although the epithelium was well preserved, because the virus is found only in low concentrations in the respiratory tract of infected animals. Hooper and Haelterman² tied off sections of the alimentary tract of piglets and returned them to the abdomen after inoculating virus. Using a different strain of virus from ours, they found highest titres in duodenum and jejunum, low titres in ileum, and no growth in stomach or colon. The apparent discrepancies with our results cannot, at the moment, be explained.

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Inhibition of Oncogenicity of Murine Sarcoma Virus (Harvey) in Mice by Interferon

HOMOLOGOUS interferon preparations have been shown to inhibit the oncogenicity of polyoma virus¹ and the evolution of several murine leukaemias²⁻⁵. Although treatment of mice with polycytidylic-polyinosinic acid, an inducer of endogenous interferon, was associated with inhibition of the oncogenicity of murine sarcoma virus, Moloney strain (MSV-M)^{6,7} exogenous interferon was reported to be ineffective in newborn BALB/c mice inoculated with this virus^{7,8}. By contrast, we report the efficacy of exogenous interferon preparations in the treatment of adult BALB/c mice inoculated with the Harvey strain of murine sarcoma virus (MSV-H).

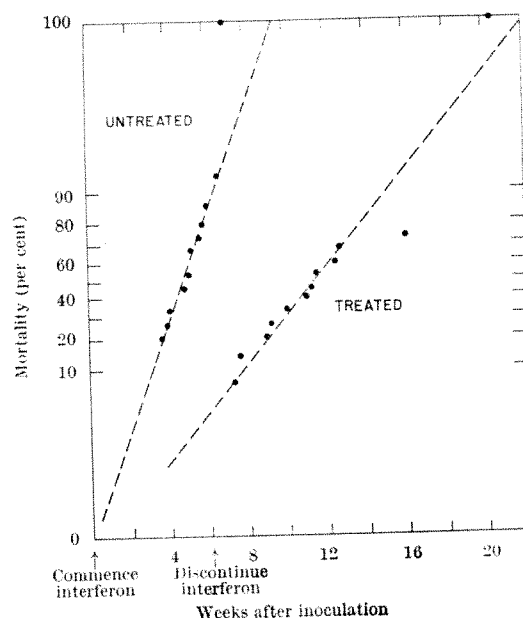


Fig. 1. Comparative cumulative mortality of BALB/c mice inoculated with MSV-H; untreated, or treated with mouse brain interferon. There were fifteen animals per group.

The MSV-H was a stock prepared from a 10 per cent mouse tumour extract supplemented in approximately equal volume with cell free supernatant fluid from a virus-producing tissue tumour cell line grown in culture⁹. This preparation had a titre of $>10^4$ TPD 50/ml. in newborn BALB/c mice which were obtained from the breeding colony of the Institut de Recherches Scientifiques sur le Cancer. The preparation and assay of concentrated mouse brain interferon have been described before¹⁰. The titre of the interferon preparations utilized was 8,000-16,000 U/0.2 ml.

Thirty 2 week old BALB/c mice were inoculated intraperitoneally with 0.1 ml. of stock virus. Fifteen mice were treated with 0.2 ml. of the interferon preparations twice daily for the next 30 days (alternately intraperitoneal and subcutaneous), and only once daily for the ensuing 10 days, at which time treatment was discontinued. Fifteen mice were not treated.

Treatment with interferon was associated with a marked delay in mortality (Fig. 1). Although all control mice were dead by the seventh week, none of the mice treated with interferon had succumbed. Nevertheless all treated mice were dead 20 weeks after inoculation of virus (14 weeks after termination of therapy). Autopsy of mice in both groups revealed the erythroblastic splenomegaly and discrete peritoneal sarcomata characteristic of disease produced by intraperitoneal inoculation of MSV-H^{9,11}.

These results demonstrated that daily treatment with concentrated mouse brain interferon preparations was associated with a significant delay in the mortality of BALB/c mice inoculated with Harvey strain of murine sarcoma virus. Similar preparations of interferon, however, were ineffective when newborn BALB/c mice were inoculated with the Moloney strain^{7,8}. This discrepancy can be explained by differences in the age of mice and viral strain utilized. Newborn mice are more sensitive to the oncogenicity of MSV than adult mice, in which the tumours commonly regress¹². The longer latent period in these mice (4-6 weeks, compared with 1-2 weeks in newborns) permitted a more prolonged course of interferon therapy. But it cannot be excluded that MSV-H transformed cells are more sensitive to interferon than cells transformed by MSV-M.

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Exogenous Interferon protects Mice against *Plasmodium berghei* Malaria

WE have shown previously that several inducers of interferon protect mice against *Plasmodium berghei* malaria and that this protection is far greater against sporozoite-induced infections than against blood form-induced infection¹⁻³. Others have reported that prolonged incubation of parasitized red blood cells (RBC) with serum containing interferon *in vitro* decreased or abolished the capacity of these cells to initiate lethal malarial infection in mice⁴. Another group found, however, that interferon had no effect on the development of malaria parasites in RBC *in vitro* (personal communication from T. Merigan). We show here that exogenous mouse serum interferon protects mice against sporozoite-induced *Plasmodium berghei* malaria.

Four week old, female, CF1 mice (Carworth Farms) were injected intravenously with 5,000 *P. berghei* sporozoites suspended in mixture 199. The progress of the infection was monitored by taking daily blood smears and counting the number of infected RBC per 10,000 RBC. The mean pre-patent period (interval between day of inoculation and first day of detectable parasitaemia) and the rate of detectable parasitaemia (number of mice developing parasitaemia/number of mice inoculated) were derived from these data. The mice were observed for 35 days. To prepare mouse serum interferon, 4 week old mice were injected intravenously with 10⁹ plaque-forming units (p.f.u.) of Newcastle disease virus (NDV), and they were bled from the retro-orbital plexus 6 to 8 h later; the serum was dialysed for 4 to 5 days against a buffer with a pH of 2 and then against phosphate-buffered balanced salt solution at pH 7 for 4 h. The interferon titre of the serum was determined by a plaque reduction method, using vesicular stomatitis virus mouse L cells; one unit of interferon activity was the amount that gave a 50 per cent reduction in plaque number⁵. Rabbit serum interferon was prepared and assayed in the same way, except that rabbit kidney cells were used. The interferon titres of the sera ranged from 1,000 to 20,000 units per ml. after dialysis. The sera were injected without further dilution.

Groups of eight to ten mice received three injections of interferon containing serum 19, 22 and 26 h after

Table 1. RELATION OF THE ANTIMALARIAL EFFECT OF INTERFERON PREPARATIONS TO THEIR INTERFERON TITRES

Source of interferon	Interferon units administered per mouse $\times 10^3$	Mortality IF*	Mortality C†	Increment in pre-patent period Δx ‡	P§	Median number of parasitized RBC per 10,000 RBC on day 6 IF	C
Mouse serum*	10.4	5/8	8/8	+1.50	<0.001	14	348
"	7.4	3/8	8/8	+1.10	<0.001	9	166
"	2.3	6/8	7/8	+1.03	<0.01	45	486
"	0.9	8/8	8/8	+0.63	<0.05	156	348
"	0.6	10/10	10/10	+0.03	>0.1	56	91
Rabbit serum	8.0	7/8	8/8	+0.04	>0.1	504	308

* IF, mice receiving preparations with interferon (0.4 ml. intravenously and 0.6 ml. intraperitoneally; the total amount injected = 1.0 to 2.0 ml.).

† C, mice receiving control serum preparations (normal mouse serum which had been dialysed in the same way as the serum with interferon). Sporozoite-inoculated mice which received no serum showed no significant difference from mice which received normal serum.

‡ Δx , mean pre-patent period of mice receiving interferon containing preparations minus mean pre-patent period of mice receiving control preparations, in days.

§ P, according to the *t* test (comparison of mice receiving interferon-containing preparations with mice receiving control preparations).

¶ Mouse serum: four different pools of mouse sera with interferon were used: the serum pool on line 4 is the same as the serum pool on line 1, but it has been diluted ten-fold.

sporozoite inoculation (Table 1). A sporozoite-inoculated control group of mice which received NDV (10⁹ p.f.u., that is, a dose which is expected to give a peak serum interferon titre of 10³ to 10⁴ units) intravenously 20 h after sporozoite inoculation was included in two experiments. An antimalarial effect was found when a dose of mouse serum interferon with an activity greater than or equal to about 1×10^3 units was injected. The degree of protection granted by mouse serum was greatest in the case of preparations with the highest titres of serum interferon. Rabbit serum with interferon had no antimalarial activity, although its interferon titre in rabbit cells was as high as the interferon titre in mouse cells of mouse serum with high antimalarial activity. The groups of mice treated with NDV had a mortality rate of 0/16, that is, NDV in dose of 10⁹ p.f.u. granted a stronger protection than any of the preparations of interferon used.

In other experiments, groups of mice received a single dose of mouse serum with interferon (5 to 10×10^3 units, partly intraperitoneal and partly intravenous). The time of the injection ranged from 3 h before to 45 h after sporozoite inoculation (Table 2). There was protection in mice injected 3 h before and 12, 21 or 37 h after inoculation and no protection in mice injected 45 h after inoculation. Mice receiving a single injection 21 h after inoculation were not protected as well as mice receiving the same amount of interferon in three divided doses between 19 and 26 h (compare Tables 1 and 2).

The results show that mouse serum with interferon exerts a significant protective effect against sporozoite-induced *P. berghei* malaria, when it is injected during the pre-erythrocytic phase of development (which ends about 42 to 48 h after sporozoite inoculation⁶). The failure to demonstrate protection when serum with interferon was injected 45 h after sporozoite inoculation suggests that interferon had no detectable effect on the

Table 2. RELATION OF THE ANTI-MALARIAL EFFECT OF MOUSE SERUM INTERFERON TO THE TIME INTERVAL BETWEEN INOCULATION OF SPOROZOITES AND INJECTION OF INTERFERON

Experiment number	Time of injection (h)*	Mortality IF†	Mortality C‡	Increment in pre-patent period Δx §	P¶	Median number of parasitized RBC per 10,000 RBC on day 6 IF	C
1	+12	4/6	6/6	+1.0	<0.01	3.5	48
	+21	3/6	5/6	+0.4	>0.1	5	30
	+37	6/6	6/6	+1.7	<0.02	4	75
	+45	5/6	6/6	+0.0	>0.1	95	75
2	-3	3/6	6/6	+0.5	>0.1	8	19
	+21	4/6	6/6	+0.4	>0.1	0.5	18
	+37	5/6	6/6	+0.5	>0.1	2	20

* Zero time = time of inoculation of sporozoites.

† IF, mice treated with mouse serum with interferon: 10,000 units per mouse (exp. 1) or 5,000 units per mouse (exp. 2).

‡ C, mice treated with control mouse serum preparations.

§ Δx , mean pre-patent period of mice receiving mouse serum preparations with interferon minus mean pre-patent period of mice receiving control serum preparations, in days.

¶ P, according to the *t* test (comparison of IF mice with C mice).

erythrocytic phase of the infection. The fact that NDV exerted a greater protective effect than the best preparation of mouse serum with interferon may be interpreted in several ways: (1) we did not use interferon preparations of sufficiently high titre; (2) the stronger protective effect of NDV is mediated by something other than serum interferon, such as intracellular interferon or an added protective effect not related to interferon; or (3) it is necessary, for maximum protection, to have prolonged, continuous production of interferon.

The facts that the antimalarial effect was demonstrated after prolonged dialysis against pH 2 buffer, that this effect was directly related to the interferon titre of the preparation and that it was species-specific provide strong circumstantial evidence that the antimalarial effect was caused by interferon present in the serum. Further support for this conclusion comes from experiments showing that interferon produced in cultures of mouse L cells with the double-stranded polynucleotide polyinosinate-polycytidylic acid⁸ also protected mice from sporozoite-induced *P. berghei* malaria (unpublished work of R. I. J. and J. V.). A final confirmation of the conclusion that interferon is indeed the active agent will require experiments with purified interferon preparations; such experiments are now in progress.

Interferon, once thought to be exclusively an antiviral agent, has now been shown to act against three other kinds of intracellular agents: *Chlamydia*⁷⁻⁹, *Toxoplasma gondii*¹⁰ and, in our experiments, a *Plasmodium* during its pre-erythrocyte phase of development.

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Inhibition of Lymphocyte Activation at High Ratios of Concanavalin A to Serum depends on Complement

WHEN certain mitogenic agents are added to lymphocytes cultured in serum, the cells are activated to transform and divide¹. A dependence of the transformation response on the ratio of mitogen concentration to serum concentration has been shown with phytohaemagglutinin², concanavalin A (our unpublished results), pokeweed mitogen and mercurous chloride³. At optimum ratios of mitogen to serum most of the lymphocytes in the culture transform, whereas at higher ratios transformation is inhibited².

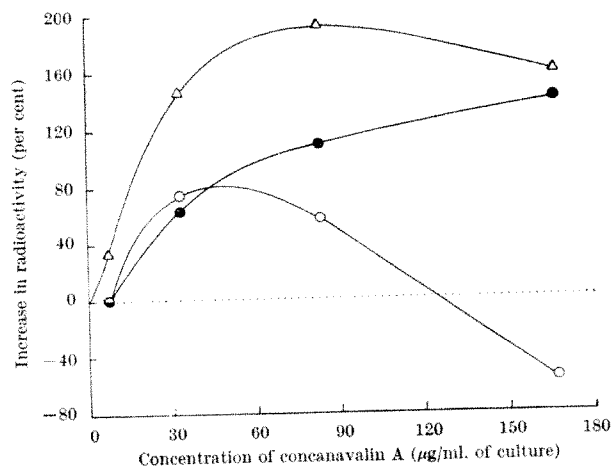


Fig. 1. Dose response curves showing the percentage stimulation by concanavalin A of the labelling with 5-³H-uridine of rat lymph node cells cultured in 20 per cent homologous serum (Δ), 20 per cent calf serum (○) or 20 per cent calf serum preheated at 56° C for 30 min (●). Cultures (1 ml.) containing 2.8×10^6 cells, 1 μ Ci of 5-³H-uridine (22.7 Ci/mmole) and 80 per cent medium 199, were incubated at 37° for 6 h in an atmosphere of air : CO₂ (95 : 5)¹⁰. Cells were pelleted by centrifugation and fixed in 95 per cent ethanol for measurement of acid-precipitable radioactivity as described before¹¹. Each point is the mean of duplicate cultures.

The activation of lymphocytes by non-specific mitogens has been suggested as a model for the study of lymphocyte activation by specific antigens⁴; one explanation of the inhibition at high ratios of mitogen to serum is that it might be analogous to the induction of specific tolerance by high antigen concentrations found *in vivo*⁵ and *in vitro*⁶. A theory of immunity based on this model has predicted that complement is involved both in inhibition at high ratios of mitogen to serum and in tolerance induction^{4,7}; there is some *in vivo* evidence for the latter⁸. Complement is a serum activity which has the properties of (i) mediating the rapid inactivation of cells; (ii) heat-lability; (iii) zymosan-lability, and (iv) lability when stored for a long time. The response of chicken lymphocytes in homologous serum to phytohaemagglutinin is enhanced if the serum is preheated, but dependence of this effect on the concentration of mitogen has not been shown⁹. We report here that the inhibition obtained at high ratios of concanavalin A to serum is dependent on a serum activity which is destroyed by preheating serum at 56° for 30 min. Other experiments (our unpublished results) have shown that the serum activity is removed by absorption with zymosan and by prolonged storage. The activity is therefore presumed to be complement.

Rat lymph node cells were cultured with 5-³H-uridine in medium 199 and 20 per cent fresh homologous or calf serum¹⁰. Concanavalin A stimulated the labelling of acid-precipitable material with tritium when measured¹¹ after 6 h in culture. Fig. 1 shows dose response curves of the percentage stimulation of labelling produced by different concentrations of concanavalin A. The cultures contained either fresh homologous serum, calf serum, or calf serum which had been subjected to a temperature of 56° C for 30 min before use. In unheated homologous serum, labelling increased to a maximum as concentrations of concanavalin A increased, after which there was a small and variable degree of inhibition. In unheated calf serum there was considerable inhibition at high concanavalin A concentrations; labelling was often depressed below that of control cultures containing no mitogen. There was no such inhibition in cultures containing preheated calf serum, and the labelling in the presence of high concentrations of concanavalin A approached the maximum labelling obtained with homologous serum. Maximum labelling in preheated homologous serum did not differ in any consistent manner from maximum labelling in unheated homologous serum.

Our data show that inhibition of lymphocyte activation at high ratios of concanavalin A to serum is dependent on a serum activity which can be destroyed by preheating serum at 56° C for 30 min, zymosan absorption and prolonged storage. We therefore presumed that this activity is due to complement. The reason why we found profound inhibition of activation and heat lability with calf serum but not with fresh homologous serum is discussed in ref. 12. Explanations of these findings include a model in which lymphocyte activation by non-specific mitogens is seen as analogous to lymphocyte activation by specific antigen⁷. If the model is good our findings predict that complement will be shown to be involved in the inhibition-in-excess effect of specific antigens.

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Chimaerism after Introduction of Lymphocytes into Normal Mice

THE lymphocyte population of mice can be thought to consist of at least two cell types¹, of which one, the T cell², derives from the thymus and which seems to be the only type of cell which can divide in response to phytohaemagglutinin (PHA)^{3,4}. The experiments described here involve the introduction of syngeneic but cytologically marked thymus grafts or lymphocyte suspensions into intact unirradiated mice and the subsequent analysis of PHA responsive (T) cells in the blood. Two assumptions are made in the interpretation of the results: first, that all cells dividing in response to PHA are T cells, on evidence reported before⁴, and second, that most T cells are responsive to the mitogenic effects of PHA. This seems likely, for the proportion of peripheral blood lymphocytes which responds to PHA (approximately 70 per cent⁵) is nearly the same as the proportion of θ positive cells (θ positivity of lymphocytes has been equated with thymic derivation⁶). The existence of a small percentage of PHA non-responsive T cells would not in any case invalidate the conclusions which will be drawn.

Our results indicate that cells derived from a neonatal thymus lobe implanted into an otherwise untreated

Table 1. EXPERIMENTAL PROCEDURE FOR SHOWING THE APPEARANCE OF CELLS DERIVED FROM IMPLANTED NEONATAL THYMUS LOBES IN THE BLOOD OF OTHERWISE UNTREATED CBA/Lac MICE, COMPARED WITH THAT IN THYMECTOMIZED, OR IRRADIATED, OR THYMECTOMIZED AND IRRADIATED MICE

Operation	Age of mice (weeks)	1	2	3	4
Thymectomy	8	—	+	—	+
Irradiation (850 r.) + 5×10^6 CBA/Lac bone marrow cells	9	—	—	+	+
Implantation of neonatal CBA/H.T6T6 thymus lobe	9	+	+	+	+
Exsanguinated for PHA culture and cytological analysis	19	+	+	+	+

adult mouse become a small but significant part of the T cell pool of the recipient. A similar phenomenon is observed after injection of large numbers of free lymphocytes. We have also shown that introduced T cells persist in the circulation for a relatively long time.

In the first experiment, four groups of CBA/Lac mice were treated according to the schedule indicated in Table 1. All received one thymus lobe from a newborn CBA/H.T6T6 mouse (implanted under the kidney capsule), and 70 days later they were exsanguinated and the peripheral blood lymphocytes isolated for culture with PHA. Metaphase preparations were made from the cultures which were collected after 3 days of incubation, colcemid having been added 16 h before collection. Details of the method have been given elsewhere⁴.

The percentage of CBA/H.T6T6 cells dividing in cultures from each of the four groups is given in Fig. 1. Explanation of the responses observed relies chiefly on what is known about the T cells which divide in response to PHA in a group 4 mouse. Sixty per cent of the response was by cells of certain thymic (T6T6) origin. Of the dividing Lac cells, it has been shown that some 10 per cent are a 50:50 mixture of host T cells which have survived 850 r. and of T cells present in the therapeutic bone marrow inoculum given at the time of irradiation. The remaining 30 per cent of Lac cells are thought to have become PHA-responsive after "processing" by the thymus graft. Evidence that this is so has been presented elsewhere⁴.

The reduction in the relative contribution to the total T cell pool by T6T6 cells in group 3 can be attributed to the presence of a thymus in the irradiated animal. The regeneration of this thymus *in situ* is likely to involve exclusively Lac cells, for the only source of T6T6 cells is the thymus graft, and the migration of stem cells from one thymus graft to another has not been observed (unpublished results of H. S. Micklem, A. J. S. D. and V. W.). Therefore, at the same time as T cells of T6T6 type are being manufactured and liberated by the thymus graft, T cells of Lac type are generated by the host thy-

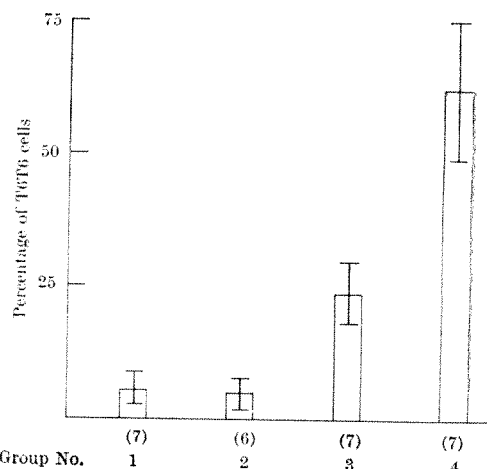


Fig. 1. The mean percentage of CBA/H.T6T6 cells mitosing in peripheral blood cultures of four groups of CBA/Lac mice treated as indicated in Table 1. The number of mice in each group is given in parentheses.

mus, and reconstitution of the irradiation-depleted, circulating T cell pool in these mice will be brought about initially by both Lac and T6T6 cells, unlike the reconstitution in group 4 which is initially only by T6T6 cells. The relative contribution to the total response by T6T6 cells in group 3 is therefore expected to be less than in group 4. The magnitude of the difference is a measure of the extent to which the irradiated thymus *in situ* and the thymus graft have performed relative to each other. It can be seen that the irradiated thymus is at least as efficient as, if not more so than, the unirradiated graft.

The situation in groups 1 and 2 differs from that in other groups chiefly because the circulating T cell pool has not been depleted by irradiation, so that any T6T6 cells leaving the thymus graft have to compete for a place in an already full pool. The assumption is that in normal circumstances a homeostatic mechanism regulates the upper size of the lymphocyte pool. This is a widely accepted assumption based in part on the observation that implantation of large numbers of thymus grafts into mice had no long term effect on their peripheral blood lymphocyte levels⁶. A further difference between implantation of a graft into irradiated and unirradiated mice is that the division of T6T6 cells in the graft ceases 20 days after implantation in normal or thymectomized mice⁷, compared with 25 days in irradiated mice⁸. Whatever the reason for this difference it could mean that the output of T6T6 cells from the graft was much more limited when their apparent regeneration time was shorter. Clearly in these circumstances the relative contribution of graft derived cells to the total response in unirradiated mice is likely to be smaller. The apparent lack of any effect of thymectomy in unirradiated mice is an interesting indication that in the absence of the thymus, T cells persist in the circulation for a long time relative to the 20 days in which T6T6 cells are likely to be emerging from the graft⁷. If this were not so, the thymectomized group would be expected to have a higher proportion of T6T6 cells in their circulation than the non-thymectomized mice.

This preliminary observation on the long term persistence of T cells has been extended by thymectomizing CBA/Lac mice, one group 68 days and the second group 8 days before they received a CBA/H.T6T6 thymus graft. The two groups were exsanguinated 71 days later and the proportion of T6T6 cells responding to PHA

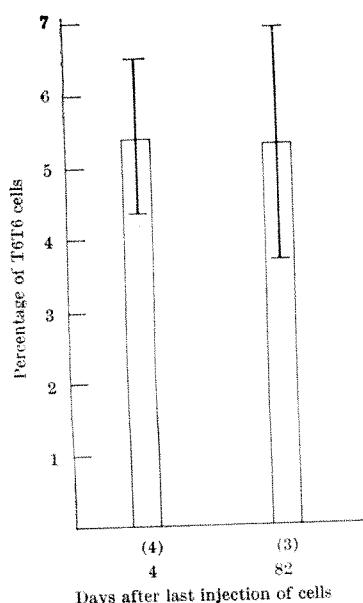


Fig. 2. The mean percentage of CBA/H.T6T6 cells mitosing in response to PHA in blood cultures of adult CBA/Lac mice given ten weekly injections of 3×10^7 CBA/H.T6T6 thymocytes. The number of mice at each time is given in parentheses.

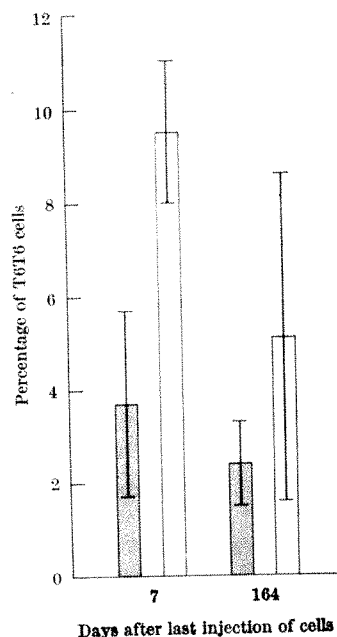


Fig. 3. The mean percentage of CBA/H.T6T6 cells induced to divide in response to oxazolone in axillary lymph nodes of adult CBA/Lac mice given three injections of 3×10^8 CBA/H.T6T6 thymocytes (shaded columns) or spleen cells (white columns). Each column is the mean of values obtained from three mice.

was determined for each mouse. No difference was found between the two groups; the percentage of T6T6 cells found dividing in mice thymectomized 68 days before grafting was 2.8 ± 1.5 per cent ($n=6$) and in those thymectomized 8 days previously was 3.7 ± 1.4 per cent ($n=5$). This confirms by another parameter evidence already obtained that thymectomy alone has no effect on the apparent magnitude of the circulating PHA-responsive T cell pool for at least 60 days⁴.

In the second experiment, one group of intact 8 week old CBA/Lac mice was given a series of ten weekly intravenous injections of 3×10^7 washed thymocytes obtained from the thymuses of 4 week old CBA/H.T6T6 mice. Four days after the last injection four animals were exsanguinated and their lymphocytes cultured for cytological analysis. Three remaining animals were killed 82 days later for the same purpose. The results (Fig. 2) indicate that some of the injected cells became part of the circulating T cell pool, and were retained at apparently the same level for a relatively long time.

In a further similar experiment, 3×10^8 CBA/H.T6T6 thymocytes or spleen cells were administered intravenously in three equal injections delivered at 2 day intervals to two groups of intact adult CBA/Lac mice. Persistence of the injected cells was detected by means of stimulation of the axillary lymph nodes with oxazolone, a skin sensitizing agent capable of inducing a delayed hypersensitivity reaction⁹, and of causing mitosis of thymus derived cells¹⁰. Oxazolone was given to both groups 7 days after the third injection and the lymph nodes were removed for analysis 3 days later. A second analysis was performed 157 days after the first, and the percentage of CBA/H.T6T6 cells dividing in the lymph nodes at each time is indicated in Fig. 3. The results are similar to those obtained by stimulation of blood lymphocytes with PHA, in so far as there was a long term persistence of the injected cells. But there seems to have been a slight diminution with time in the contribution to the response by the donor cell population. The mean contribution of donor cells in the group injected with spleen cells was greater at both times than in the thymocyte injected group, a possible indication of a greater proportion of responsive cells in the spleen than the thymus.

It seems remarkable that the injection of so many cells caused no greater dilution of the recipient's T cell population. But because (a) the proportion of fully thymus-processed cells in the lymphocyte suspensions and (b) the mechanisms whereby an animal (presumably) maintains a relatively constant lymphocyte pool size are unknown, it is difficult to calculate accurately the number of T cells in the adult mouse. Further experiments, using various lymphoid tissues as the source of lymphocytes, may help to resolve the problem.

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Is there a Unique Conformation of Cholinergic Ligands responsible for Muscarinic Activity?

CHOTHIA¹ has proposed a structure activity relationship for the muscarinic response of acetylcholine. In deriving the model responsible for optimum activity, reports on the pharmacological testing of a number of conformationally constrained acetylcholine analogues were neglected.

A principal test of any structure activity relationship is the synthesis and testing of analogues of the ideal substrate. Here we present some structural and pharmacological data which do not seem to follow the "cause and effect" relationship presented by Chothia¹.

For high muscarinic activity a molecule should be able to adopt an arrangement for the N⁺-C-C-O atoms "between positive synclinal and positive anticlinal" such that the "methyl side of acetylcholine is preserved while the carbonyl side is blocked"¹. The methyl side refers to the position of the molecule which connects CH₃(1) and the acetoxy methyl group (see Fig. 1). The structures of *threo*- and *erythro*- α - β -dimethyl-acetylcholine iodides have been determined³ and these molecules tested for muscarinic activity⁴. The *threo*- α (S)- β (S)-stereoisomer can easily adopt the conformation shown in Fig. 1. The N⁺-C-C-O conformational angle is +143 (ac) for the *threo*- α (S)- β (S) isomer and +76 (sc) for the *erythro*- α (R)- β (S)-enantiomer. Because activation of the muscarinic receptor requires that the methyl side be clear to attack the receptor surface¹, one would expect the *threo*-isomer to be a more potent muscarinic agonist than the *erythro*-molecule. The opposite, however, is found⁴: the apparent activity of the *threo*-compound is negligible. With regard

to acetylcholinesterase hydrolysis of these molecules, the *threo*-molecule degraded at a much greater rate than the *erythro*-analogue⁴.

Another example that has been studied⁴ is 3(axial)-trimethylammonium - 2(axial) acetoxy - *trans* - decaline methiodide. This molecule is very similar to the *threo*-analogue both structurally and pharmacologically. The N⁺-C-C-O conformation angle is +147 (ac) for the 3(S)-2(S) enantiomer (see Fig. 2). It thus does not seem to comply with the Chothia model¹.

The thiol and selenol analogues of acetylcholine should be able to adopt an overall conformation that is quite similar to the muscarinic model proposed⁵. Models based on the van der Waals radius for sulphur of 1.65 Å bear this out⁶. The muscarinic activities of these molecules differ greatly^{7,8} from acetylcholine. The relative importance of electronic distribution versus conformation with regards to biological activity has already been alluded to⁵.

Chothia¹ points out that "the principal limitation on the supporting structure is that it should not sterically hinder the interaction of the essential structure with the receptor", and he further assumes that there is only one binding site responsible for the initiation of a pharmacological response. That the validity of these assumptions is open to question is shown by the recent work of Moran and D. J. T.^{2,9}, who, from comparative studies of agonist interaction, have proposed a dual mode of muscarinic agonist binding in which a common anionic site is flanked by two binding loci for polar and nonpolar side chains of quaternary trimethylammonium ligands.

The essential structure (both conformationally and electronically) remains to be formulated with sounder reasoning. If one wishes to speculate about the structural requirements necessary to produce a muscarinic response, account should be made of the probable multiplicity of ligand binding modes at the receptor. It may be that the "hydrophobic" surface requires molecules in the anticlinal conformation (that is, the conformation about the ethylene bridge of the choline residue) and in the other region the synclinal rotamers are effective. The orientation of methyl groups on acetylcholine analogues might thus have two effects: to promote binding to the "hydrophobic" receptor surface and to prevent interaction with the surface by steric repulsion. It might also be noted that the general concept of multiple modes of ligand interaction at a single catalytic site receives strong support from studies on a number of enzyme systems, including acetylcholinesterase¹⁰, lysozyme¹¹, trypsin¹² and carboxypeptidase¹³.

An extension of this analysis will be published in the proceedings of a forthcoming symposium¹⁴.

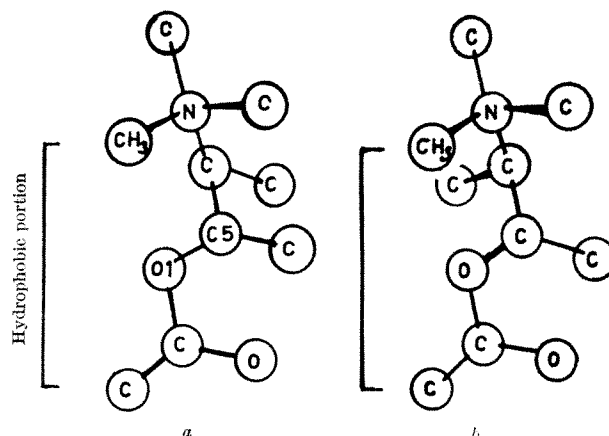


Fig. 1. General views of the structures of (a) *threo*- α (S)- β (S)-dimethyl-acetylcholine and (b) *erythro*- α (R)- β (S)-dimethylacetylcholine. The region denoted as the "methyl side"—the hydrophobic portion of the molecule—is labelled.

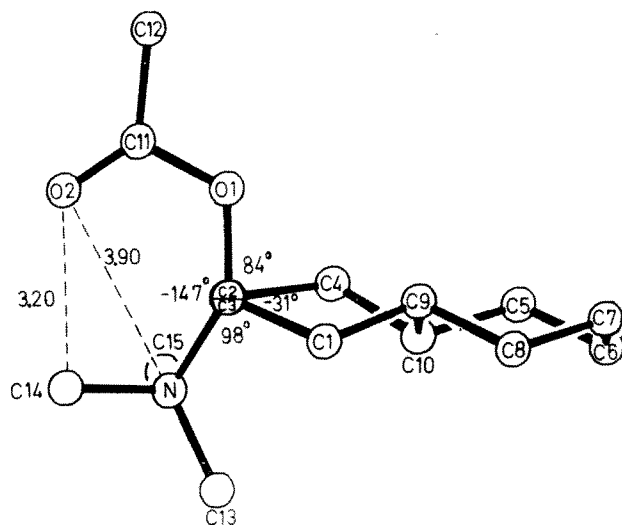


Fig. 2. 3(Axial)-trimethylammonium-2(axial) acetoxy-*trans*-decaline methiodide as seen down the C(2)-C(3) bond. The 3(*R*)-2(*R*) enantiomorph is illustrated.

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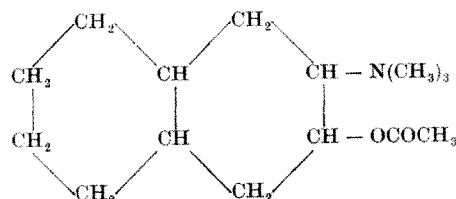
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Structure Activity Relationships of some Muscarinic Agonists: a Reply to Shefter and Triggle

IN essence, the criticism by Shefter and Triggle¹ of my model² for the interaction of acetylcholine, $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{OCOCH}_3$, with muscarinic receptors is that it does not explain the muscarinic potency of some analogues of acetylcholine. I believe that my model does quite simply account for the activity of these compounds. As background information for my model, I made the general statement that muscarinic agonists are observed to have the $\text{N}^+-\text{C}-\text{C}-\text{O}$ torsion angle between the positive synclinal and positive anticlinal³ (Fig. 1a). Shefter and Triggle do not quote any potent agonists for which this observation is untrue and I briefly show how it can be used to explain the muscarinic activity of the compounds they mention.



(a) Acetylselenocholine and acetylthiocholine, $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2(\text{Se or S})\text{COCH}_3$, are weak muscarinic³ agonists for two reasons. First, the $\text{N}^+-\text{C}-\text{C}-\text{Se(S)}$ torsion angle is antiperiplanar ($\sim 180^\circ$), in both crystals and solution⁴ (Fig. 1b). The conformation of $\text{N}^+-\text{C}-\text{C}-\text{Se(S)}$ between synclinal and anticlinal is energetically unfavourable because of the larger van der Waals radii of sulphur (1.8 Å) and selenium (2.0 Å) compared with that of oxygen (1.4 Å) and to their lower electronegativities. Second, the difference between the observed $\text{C}-\text{Se(S)}$ bond lengths and the $\text{C}-\text{Se(S)}-\text{C}$ bond angles⁵ and the observed $\text{C}-\text{O}$ bond lengths and the $\text{C}-\text{O}-\text{C}$ bond angle in acetylcholine means that these compounds are not isostructural with acetylcholine. The position of the methyl group C7 which is very important for muscarinic activity² is moved ~ 0.95 Å relative to its position in acetylcholine (Fig. 2).

(b) *Erythro*- α,β -dimethylacetylcholine iodide: $(\text{CH}_3)_3\text{N}^+\text{CH}(\text{CH}_3)\text{CH}(\text{CH}_3)\text{OCOCH}_3$. This is a moderate muscarinic agonist⁶. The observed $\text{N}^+-\text{C}-\text{C}-\text{O}$ torsion angle ($+76^\circ$) of the $\alpha(R)-\beta(S)$ enantiomer⁴ is in the region necessary for muscarinic activity (Fig. 1c). Steric hindrance by the α -methyl group reduces this activity as it does in acetyl- α -methylcholine^{2,7}. The observed potency is very probably enhanced by its low rate of hydrolysis by acetylcholinesterase⁸.

Threo- α,β -dimethylacetylcholine iodide: Shefter and his colleagues have shown that in crystals of the iodide the $\text{N}^+-\text{C}-\text{C}-\text{O}$ torsion angle of the $\alpha(S)-\beta(S)$ enantiomer is

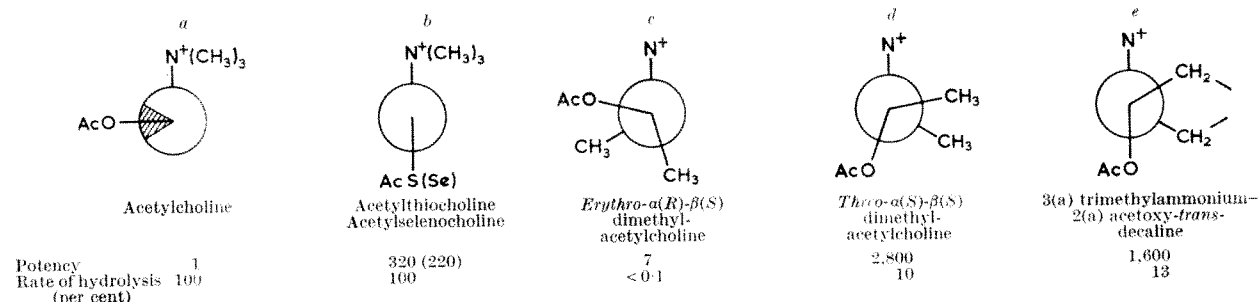


Fig. 1. Newman projections of the $\text{N}^+-\text{C}-\text{C}-\text{O}$ conformation for: a, molecules active at muscarinic receptors—the shaded area shows the region in which values are found for potent agonists²; b, acetylthiocholine and acetylselenocholine in crystals and solution⁴; c, *erythro*- $\alpha(R)-\beta(S)$ dimethylacetylcholine in crystals of the iodide⁴; d, *threo*- $\alpha(S)-\beta(S)$ dimethylacetylcholine in crystals of the iodide⁴; and e, 3(axial)-trimethylammonium-2(axial) acetoxy-*trans*-decaline methiodide. Muscarinic potencies^{2,3} are given as the number of molecules of the racemate equivalent to one molecule of acetylcholine. The rate of hydrolysis is that by acetylcholinesterase^{2,8}.

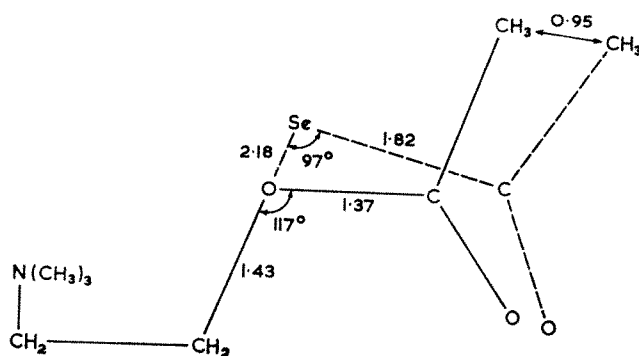


Fig. 2. Superposition of the choline residues of acetylcholine and acetyselenocholine shows that the methyl groups differ by 0.95 Å in their relative positions. The C-Se bond lengths and the C-Se-C bond angle are those found in crystals of the iodide⁶. The bond lengths and angles of the acetoxy group are the accepted values and are consistent with those found in cholinergic molecules.

nearly antiplanar (Fig. 1d) and $\tau\text{C-C-O-C}$ is 95° . For muscarinic activity the value of $\tau\text{N}^+-\text{C-C-O}$ must be reduced and $\tau\text{C-C-O-C}$ increased until it is antiplanar. Steric repulsion between the α and β methyl groups and electrostatic attraction between the carbonyl oxygen and quaternary nitrogen means that only a very small proportion of molecules will have the required conformation. These few molecules give this substance its observed low muscarinic activity.

(c) 3(axial-Trimethylammonium-2(axial) acetoxy-*trans*-decaline methiodide. The covalent structure of this compound holds the $\tau\text{N}^+-\text{C-C-O}$ rigidly antiplanar (Fig. 1e), and the compound is therefore relatively inactive. A potency⁶ of 1,600 is not very significant because "almost any compound with a quaternary nitrogen has some stimulant or inhibitory activity at cholinergic receptor sites"⁸.

Elsewhere we are describing more precisely the requirements for the structure of muscarinic agonist (R. W. Baker, C. H. C., P. J. Pauling and T. J. Petcher, manuscript in preparation).

I thank Dr Peter Pauling for discussion and Mr John Cresswell for the drawings.

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Calcium Binding, Quantum Yield, and Emitting Molecule in Aequorin Bioluminescence

THE protein aequorin, from the jellyfish *Aequorea*, emits bluish light in aqueous solution on addition specifically of calcium or strontium; the necessary energy of more than 60 kcal/mol at the peak wavelength is evidently provided by an intramolecular reaction which is accom-

panied by a change in absorption spectrum and yields a blue fluorescent reaction product^{1,2}. The extreme sensitivity to Ca^{2+} , much more than to Sr^{2+} , provides a basis for quick microdetermination of Ca^{2+} in biological fluids³ and has been used in detecting the relation of Ca^{2+} to contraction of single muscle fibres^{4,5} and to activity of mitochondria⁶. The chief properties of aequorin, including the molecular weight (m.w.) of 30,000 by sedimentation, diffusion or 32,000 by amino-acid composition, have been reported⁷. The kinetics of the light-emitting reaction have also been studied⁸.

We prepared aequorin by an improved procedure which increased the yield to 17 per cent, rather than the previous 12 per cent of the initial brei⁷. Thin circumoral rings were excised from specimens by machine⁹ and transferred to cold saturated $(\text{NH}_4)_2\text{SO}_4$ containing 0.05 M EDTA at pH 6. By violently shaking the container the photogenic organs were dislodged and the active, particulate material was filtered out through rayon gauze. After extraction in 0.05 M EDTA, the dissolved aequorin was purified as described before⁷. The product showed a single, sharp band in polyacrylamide gel disc electrophoresis, coinciding with a band of luminescence activity, which was much smaller than in the original material, in spite of efforts to minimize inactivation, for example, by not using persulphate and by maintaining a low temperature of about 3°C .

The quantum yield (photons/mole of protein) of our material was 0.23 at 25°C as reported before⁷. At 5°C the yield was 0.29 which could be increased to a maximum of 0.37 by including 1 mM *n*-heptyl alcohol in a buffered reaction mixture, pH 7.3. At 25°C the yield was 0.27 with the alcohol instead of 0.23 without. Because *n*-heptyl alcohol did not significantly increase the quantum yield in crude extracts, but only after considerable loss of luminescence activity in the process of purification, it seems that an activating substance, analogous to *n*-heptyl alcohol, is present in crude extracts and perhaps functions in living specimens.

The product of the bioluminescence reaction, previously referred to as the "blue fluorescent protein", is designated here "BFP-3Ca", for a ratio of 3.2 Ca/mole of protein was found by analysis with calcium acetate labelled with ^{45}Ca . This protein was prepared by dropwise addition of 10 mM of the calcium acetate solution, pH 7.3, to 1 ml. of aequorin solution until no more light was given off, followed by gel filtration through a 1×15 cm column of 'Sephadex G-25' (fine) equilibrated with 10 mM Tris-HCl buffer, pH 7.3 at 0°C .

Titration of BFP-3Ca with EDTA reduced the fluorescence to approximately half, whereas titration with Ca^{2+} did not affect fluorescence (Fig. 1A). The reduced fluorescence was then completely restored to the original by reverse titration with Ca^{2+} . Three independent titrations demonstrated that the decrease in fluorescence corresponded to an addition of 2.0 ± 0.1 moles of EDTA per mole protein, with the decrease beginning at 0.2 mole of EDTA per mole of protein (by extrapolation). These data indicate that (a) 2Ca, within the 3.2Ca contained in BFP-3Ca, are involved in the reversible change in fluorescence; (b) the association constant k_a for one of these two calciums is approximately $7 \times 10^6 \text{ M}^{-1}$, as estimated from the curved portion of the line sloping down from the top horizontal line in Fig. 1A; while the k_a for the other (first) calcium bound is probably considerably greater; (c) the blue fluorescent protein when bound with exactly 3Ca is fully fluorescent, and (d) BFP-3Ca contains an excess of 0.2 mole of Ca per mole of protein. Accordingly, we designate as BFP-Ca the EDTA titration product of BFP-3Ca, having the reduced fluorescence.

The calcium in BFP-Ca could be removed by addition of more than 30 moles of EDTA per mole of Ca, followed by gel filtration through a column of 'Sephadex G-25' equilibrated with Tris buffer, or by simply passing the sample

through a column of 'Sephadex G-25' equilibrated with a buffer containing 10 mM EDTA. Removing the last calcium, however, always led to irreversible changes including loss of the absorption peak at 335 nm and, therewith, disappearance of visible fluorescence. This last calcium is evidently essential for preserving the characteristics of this protein.

Data from equilibrium dialysis of BFP-3Ca in ^{45}Ca -labelled calcium acetate solution (Fig. 1B), in spite of the small number of the points, clearly indicate that (a) at least 2, probably more, different association constants are involved in BFP-3Ca, (b) the first few calciums are bound very tightly, and (c) at high concentrations of calcium one molecule of protein can bind more than 20Ca^{2+} (16Ca^{2+} in 1 mM calcium acetate solution).

The spectral distribution of the fluorescence emission of BFP-3Ca is identical to that of bioluminescence (Fig. 2, λ_{max} 469 nm), but differs significantly from the fluorescence of BFP-Ca (λ_{max} 480 nm). This evidence strongly suggests that excited BFP-3Ca is the emitter in bioluminescence, assuming that BFP-3Ca contains exactly 3Ca, even though more calciums, inert in bioluminescence, may

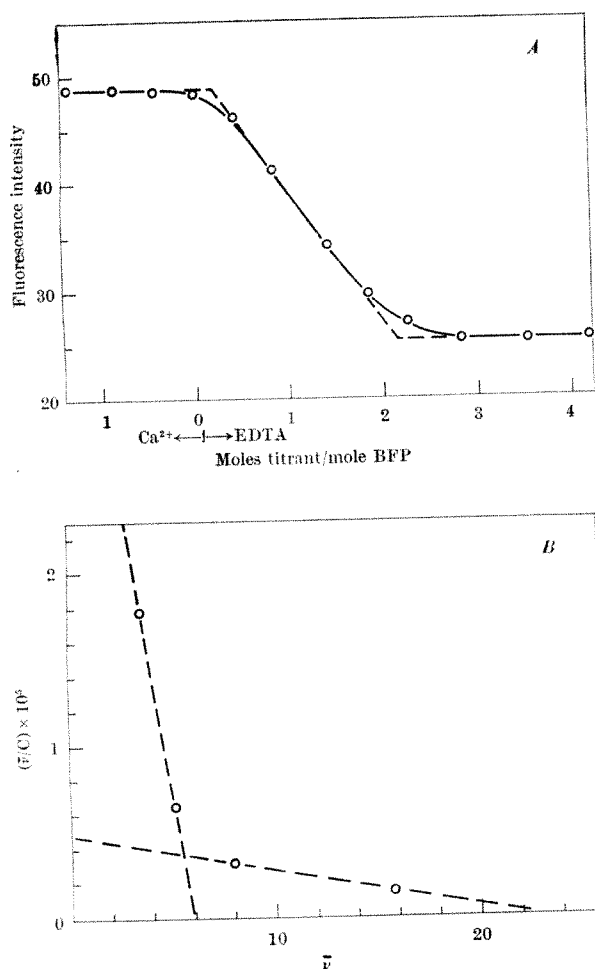


Fig. 1. A, Fluorometric titration of BFP-3Ca with Ca^{2+} or EDTA; B, scatchard plot of equilibrium dialysis data for Ca^{2+} -BFP-3Ca system. All solutions were prepared with 10 mM Tris-HCl buffer, pH 7.3, and protein concentrations determined on the basis of $E_{1\text{ cm}}^{1\%}$ at 280 nm = 26.0, m.w. = 32,000. For A, 0.65 mg of protein in 1 ml. of buffer was titrated with 1 mM of the reagents at 0°C, and fluorescence was measured at 470 nm with excitation at 340 nm on an Aminco-Bowman spectrophotofluorometer using a 3 mm square cell at 25°C. For B, aliquots of 0.95 mg of protein in 1 ml. of buffer were dialysed against 100 ml. of various concentrations (10^{-3} M – $2 \times 10^{-4}\text{ M}$) of a solution of calcium acetate labelled with ^{45}Ca (^{45}Ca obtained as $^{45}\text{CaCl}_2$, 10 mCi/mg, 1 mCi/ml.) at 3°C for 40 h. The radioactivities of working solutions were adjusted so that the 0.05 ml. samples used for counting contained 0.01–0.05 μCi ; $\bar{\nu}$, the ratio of moles of Ca bound per mole of protein; C, molar concentration of unbound Ca.

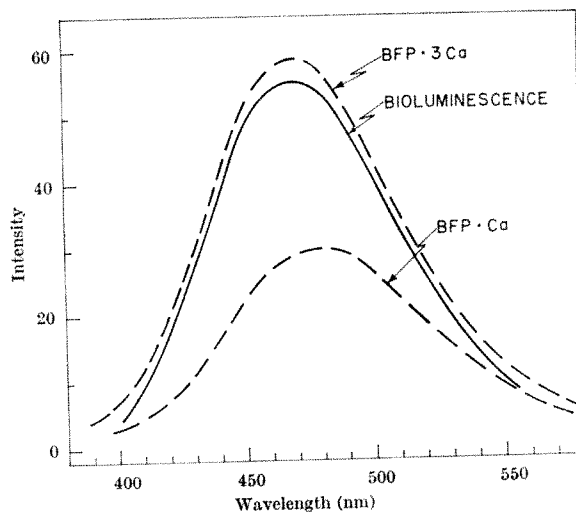
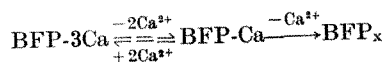
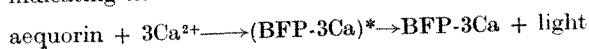


Fig. 2. Bioluminescence spectrum of aequorin, and fluorescence emission spectra of BFP-3Ca and BFP-Ca on excitation at 340 nm, measured with an Aminco-Bowman spectrophotofluorometer equipped with a Hamamatsu R-136 photomultiplier tube using a 3 mm square cell, at 25°C. The molar concentrations were the same for BFP-3Ca and BFP-Ca. Buffer: 10 mM Tris buffer, pH 7.3.

be bound relatively loosely to the protein. This leads to the following reaction scheme, BFP_x representing an irreversibly altered form of the protein and the asterisk indicating the excited state:



It is reasonable to believe, pending further evidence, that the fluorescent substances previously isolated⁷ from aequorin

AF-350 ($\lambda_{\text{fl max}}^{\text{H}_2\text{O}}$ 442 nm) and AF-400 ($\lambda_{\text{fl max}}^{\text{H}_2\text{O}}$ 462 nm)

have the same skeleton as the fluorescent chromophore in BFP-3Ca.

Finally, the foregoing evidence indicates that, in BFP-3Ca, most calcium is bound tightly enough to interfere with turnover, a circumstance which requires due consideration whenever aequorin is used in a study of extremely small concentrations of calcium.

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Infrared Absorption Spectrum of Water adsorbed on α -Helical Synthetic Polypeptides

I REPORT here some observations on the polarized infrared absorption spectrum of water adsorbed on orientated synthetic polypeptide films. The results suggest that the water molecules are adsorbed at specific sites and orientations with respect to the substrate molecules.

Orientated films of high molecular weight synthetic polypeptides in the α -helical conformation were prepared by collapsing to one end of a Langmuir trough monolayers spread on the water surface. The method used to spread the monolayers and to remove the collapsed film has been described previously^{1,2}. Sufficient monolayers were spread to give a specimen with an absorbance of 1.5 or greater for the amide I band. Films were mounted on barium fluoride plates and dried to a predetermined humidity. Where necessary, scatter of radiation from the surface of the specimen was reduced by allowing a drop of chloroform or benzene to flow across the surface so that it softened and became compacted. This treatment improved the quality of the spectra and probably modified the crystallinity of the specimen, but was not essential to see the main features described. Because the OH-stretching band lies close to the amide A (NH-stretching) band, it is helpful (but not essential) to reduce the NH absorption by using N-deuterated polymer and spreading the monolayers on 0.01 M HCl on which the back exchange of deuterium is slow¹.

High molecular weight specimens of poly-D-alanine, poly- γ -ethyl-L-glutamate and poly- γ -methyl-L-glutamate have been examined with particular reference to the 2,000–4,000 cm^{-1} spectral range. Although the strength and shape of the water absorption band varies from one polymer to another, three main features are common to all the polymers: (1) the OH absorption is not centred about 3,400 cm^{-1} as in liquid water, but is displaced to around 3,500 cm^{-1} ; (2) the band is clearly made of two or three components; (3) overall, the band exhibits marked perpendicular dichroism. Fig. 1 illustrates these features for poly- γ -methyl-L-glutamate, together with the NH and N²H stretching bands (about 3,300 and 2,450 cm^{-1}), which

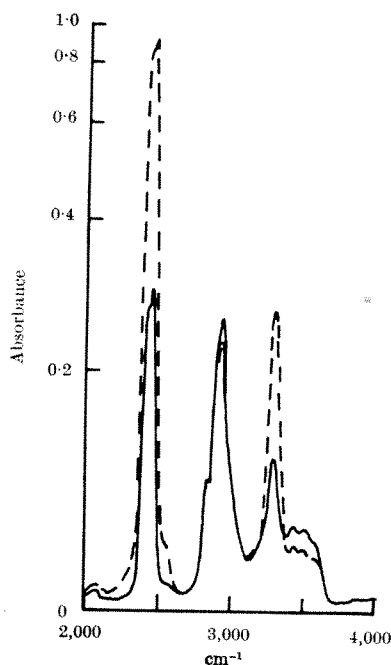


Fig. 1. Infrared absorption spectrum of a partially N-deuterated film of poly- γ -methyl-L-glutamate, 30° C, approximately 91 per cent relative humidity. Full line: electric vector perpendicular to direction of orientation; broken line: electric vector parallel.

are of opposite dichroic character to the water band, and the CH bands. The water band has components at about 3,440, 3,510 cm^{-1} and 3,570 cm^{-1} . Poly- γ -ethyl-L-glutamate has three similar bands and poly-D-alanine has a band at about 3,470 cm^{-1} with a shoulder at 3,530 cm^{-1} .

Because the frequency of the water absorption band is roughly mid-way between that of the free and hydrogen-bonded OH stretching frequencies it is probable that the hydrogen bonding component of the binding is weak. The dichroism and splitting of the band into two or three components is good evidence that the molecules are in specific orientations and sites with respect to the polymer. If the band is attributed mainly to the OH antisymmetrical stretching vibration (normally the strongest), which has a transition moment parallel to the line drawn through the hydrogen atoms, the molecules are orientated on average with this direction fairly close to planes drawn perpendicular to the helix axis. An estimate of the angle can be made by using the direction of the NH and N²H transition moments to give a measure of the orientation of the polymer. The usual model for fibre orientation is assumed with a fraction of perfectly orientated polymer and the rest disorientated³, and the water is taken to be distributed in proportion over both fractions. This leads to the H–H direction being within about 25° to the plane perpendicular to the axis of the helix.

Weak perpendicular dichroism of the same water band has been observed in hydrated α -keratin by Bendit⁴, who found the frequency to be 30–40 cm^{-1} higher than in liquid water. Although the spectrum was very similar to that of an equivalent thickness of liquid water, it is probable that the observations are closely related to the present work.

A tentative explanation of these observations, consistent with the views of Bendit for keratin, is that the water molecules orientate with a hydrogen atom directed towards the peptide oxygen, possibly forming a weak hydrogen bond to it, with the H–H direction of the water molecule within 25° to the plane perpendicular to the helix axis. In this situation it is probable that the water dipole will interact strongly with the peptide dipole. This orientation would account for the positive sign of the surface potential when a monolayer of α -helices is spread on water¹, if on a clean water surface the hydrogen atoms are normally directed downwards⁵. In the case of the glutamate polymers an additional interaction may occur between the water and the side chain carbonyl.

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Involvement of Articular Cartilage in a Linear Relaxation Process during Walking

A KNEE joint consists essentially of a pair of articulating bone surfaces lined with cartilage; the latter is swollen with an aqueous solution of protein and salts and contained within a sac (synovial membrane). The solution to prob-

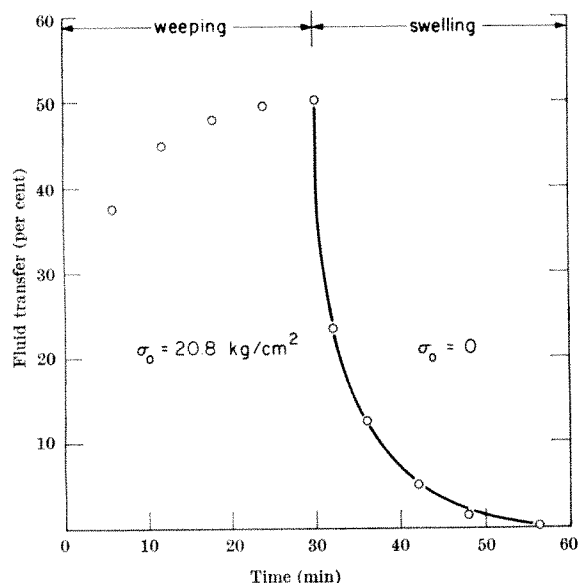


Fig. 1. A complete weeping-swelling cycle for canine cartilage in normal saline solution at 20° C. The load history is indicated. ○, Data of Edwards⁴; —, swelling behaviour predicted by equation (2) of the text using data from the weeping part of cycle.

lems of friction and wear in the normal joint has attracted attention to the properties of the bearing material, articular cartilage—a composite macromolecular gel made up chiefly of collagen and various mucopolysaccharides, together with 70–80 per cent fluid. In several *in vitro* studies, articular cartilage has been separated from bone, cut into simple geometric shapes and subjected to compressive loading. It has been repeatedly observed^{1–4} that, after the application of load, cartilage expresses a portion of its fluid within between 20 and 60 min, at the end of which the weight of transferred fluid reaches a constant level (Fig. 1). When the load is removed, fluid gradually re-enters the cartilage and swells it; eventually, the fluid content returns to its original level^{3,4}.

Several characteristics of this transfer of fluid, which is also known as “weeping” or “consolidation”, have been clarified by McCutchen, and their possible role in joint lubrication has been described². The physiology⁵ and the pathology⁶ of joint function have each been reviewed comprehensively, but no general quantitative theory accounting for the strongly time-dependent and recoverable fluid transfer in and out of cartilage subjected to an arbitrary load history seems to have been presented.

I wish to suggest that the process of fluid transfer accompanying loading and unloading of articular cartilage is a linear relaxation process with the properties of superposition and homogeneity. In particular, I propose that the percentage weight of fluid $w(t)$ transferred in or out of the cartilage at time t due to a given load applied at time τ is independent of the weight of fluid transferred due to any previous load; and that the percentage weight of fluid transferred at a fixed time after application of load is proportional to the load. According to my hypothesis, the amount of fluid transferred in or out of cartilage due to any complicated time-dependent load $\sigma(\tau)$ is therefore simply equal to the sum of the quantities of fluid transferred due to each separate increment of load. These statements can be presented compactly if $w(t)$ is equal to a linear hereditary integral of the load history $\sigma(\tau)$:

$$w(t) = \int_0^t W(t-\tau) \frac{d\sigma(\tau)}{d\tau} d\tau \quad (1)$$

where t is current time and τ is the time of application of the load. $W(t)$ is a function characteristic of the response

of an individual specimen of cartilage to the application of a constant load σ_0 suddenly applied at $\tau=0$ and will be referred to as the step response function of the individual specimen. Equation (1), a form of the well known Duhamel's integral, is supposed here to be applicable only at relatively low loads. In the most general case, to compute the percentage weight of fluid transferred at time t according to equation (1), one must first find the time-derivative of the load history, then form the product of the latter at each point in time τ and the step response function at time $(t-\tau)$, and finally integrate from $\tau=0$ to $\tau=t$. Equation (1) does not specify what form the step response $W(t)$ takes. If physiologically valid, however, this equation guarantees that a single function $W(t)$ will contain all the information necessary to predict the amount of fluid transferred in or out of an individual specimen of cartilage subjected to an arbitrary, and possibly quite complicated, loading history.

I have tested the validity of equation (1) in a preliminary way, using the data of Linn and Sokoloff³ as well as the data of Edwards⁴ shown as circles in Fig. 1 and obtained by compressing canine cartilage under a physiologically meaningful load of 20.8 kg/cm² (296 pounds/inch²) in normal saline solution at 20° C. For the simple load history used by Edwards (step loading-unloading), equation (1) predicts that the percentage weight of fluid entering the cartilage at any time during the swelling step (which commences when the load is removed) should be equal to

$$w(t) = \sigma_0 W_e - \sigma_0 W(t-t_1) \quad (2)$$

where W_e is the asymptotic limit of $W(t)$, σ_0 is the compressive stress applied suddenly at $t=0$ and t_1 is the time at which the load was removed. From Fig. 1, t_1 is 30 min and $W_e = 2.40$ per cent/kg/cm². To calculate the amount of fluid that has entered (say) 6 min after removal of the load, that is, at $t=36$ min, we would need to know $W(t-t_1) = W(6)$ which from Fig. 1 is seen to be 1.80 per cent/kg/cm². Introduction of these quantities in equation (2) gives the prediction that, at $t=36$ min, 12.6 per cent of the fluid has re-entered the cartilage. Similar predictions of points on the swelling curve, based on data from the weeping (or consolidation) curve and use of equation (2), have produced the solid curve in Fig. 1. Agreement between the experimental points and the predictions of equation (2) is satisfactory. Good agreement was also obtained between the data of Linn and Sokoloff³ and the predictions of equation (2). A definitive evaluation of the validity of equation (1) in the analysis of the weeping-swelling behaviour of cartilage *in vitro* must, however, wait until a more extensive body of data becomes available. My experience with the characteristics of other linear and non-linear relaxation processes suggests that only material systems which are rheologically well defined⁷ can be expected to obey fully equation (1). On the other hand, even if the hypothesis I have set out is correct only as an approximation, it is still possible to make use of the consequences of equation (1) to systematize the complex body of data on the time-dependent mechanical behaviour of normal and diseased cartilage and of cartilage substitutes.

During normal walking, it has been estimated^{4,8,9} that joints in the lower limbs are subjected to compressive stresses in the range 10–20 kg/cm² (about 150–300 p.s.i.) every 0.5 s. Basing my argument on the dual assumption that (a) the *in vitro* cyclical process of swelling-weeping of fluid in and out of cartilage does indeed obey equation (1) in the range of load and time scale representative of normal walking, and that (b) this fluid transfer process is an appropriate model for the major physiological events occurring in skeletal joints during walking, I am led to a simple hypothesis concerning the nature of this activity: walking amounts to square pulse compressive loading of articular cartilage which responds by expressing and

imbibing fluid in amounts that are linearly superposable and fully recoverable.

I thank Dr Eric Radin who introduced me to animal joint lubrication and wear. This work was sponsored by the National Institute of Arthritis and Metabolic Diseases.

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Low Magnesium and Phospholipid Content of Cell Walls of *Pseudomonas aeruginosa* resistant to Polymyxin

THE resistance of the pathogenic bacterium *Pseudomonas aeruginosa* to chemical inactivation is notorious. *P. aeruginosa* and related species cause problems in plant pathology^{1,2}, food spoilage³, contamination of pharmaceuticals^{4,5} and hospital cross-infection^{6,7}. We report here results relating to the mechanism of resistance of this organism which may also have wider significance for Gram-negative bacteria.

Magnesium^{8,9} and readily extractable lipid¹⁰ of the cell wall have been implicated in resistance of *P. aeruginosa*. Newton¹¹ postulated that relatively low lipid phosphorus from walls of polymyxin resistant *P. aeruginosa* may contribute to the selective action of polymyxin, the main site of action being the cytoplasmic membrane. We have investigated the whole cell and cell wall content of magnesium and readily extractable lipid of sensitive and polymyxin resistant *P. aeruginosa* NCTC 6750. It was observed that while the readily extractable lipid increased both as a percentage of the cell and of the wall of the resistant bacteria, the resistant cell walls contained greatly reduced amounts of phospholipid and magnesium compared to sensitive bacteria.

P. aeruginosa NCTC 6750 was grown in a medium consisting of 0.0143 M (NH₄)₂HPO₄, 0.0057 M NH₄H₂PO₄, 0.001 M (NH₄)₂SO₄, 0.0005 M NaCl, 0.0005 M KCl, 0.01 M glucose, 1 µg/ml. Mg²⁺ and 0.01 per cent proline. The minimum inhibitory concentration of polymyxin using inocula of 10⁶ bacteria was 16 units/ml. Serial subculture in the presence of polymyxin resulted in resistant cultures growing in 6,000 units/ml. polymyxin after three steps. The growth rates of the sensitive and resistant cultures were the same. For preparation of cell walls (envelope fraction, relatively free of cytoplasmic membrane) and whole cells, shaken batch cultures were grown in 8 l. volumes in 10 l. flasks and the medium supplemented with 0.1 µg/ml. Fe²⁺ as FeSO₄. Exponential cultures were harvested by centrifugation at an *E*₄₇₀ of 1.0 (about 0.3 mg dry weight cells/ml.). The bacteria were washed in 0.9 per cent NaCl and freeze dried. Walls were prepared by the method of Gray and Wilkinson¹² modified by increasing the period of cell disruption (at 0°–4° C) to 2 h (90–100 per cent disruption) and eliminating the wash before enzyme treatment. The walls were finally washed by centrifugation twice in 0.9 per cent NaCl to remove loosely bound cation¹³, then twice with water. Purity was verified by lack of an absorbance peak at 260 nm by wall suspensions and by electron microscopy. Phosphorus was assayed by the method of Allen¹⁴ and magnesium was determined using a Unicam SP90 atomic absorption spectrophotometer on perchloric-hydrogen peroxide digests of wall or whole cell preparations. The extraction and fractionation of readily extractable lipid into phospholipids, free fatty acids and neutral lipids were done by the method of Bobo and Eagon¹⁵ except that 0.01 per cent butylated hydroxytoluene was added as an antioxidant to the chloroform-methanol solvent¹⁶.

Thin layer chromatographic plates with about 0.1 mg phospholipid in ether were run in chloroform 72 : methanol 27 : water 1, and were developed with 10 per cent phosphomolybdic acid in ethanol and 0.5 per cent ninhydrin in acetone-butanol 1:1. Reference phospholipids were phosphatidyl ethanolamine and diphosphatidyl glycerol. The relative amounts of different phospholipids were determined by spraying to saturation with 20 per cent (NH₄)₂SO₄ in 4 per cent H₂SO₄ and heating at 110° C for 30 min, giving dark brown spots¹⁷. Plates were then scanned on a Perkin Elmer Chromoscan and each peak recorded as a percentage of the total peak area.

There was no detectable qualitative difference in the phospholipid fraction of the readily extractable lipid of sensitive whole cells and walls. The major component in each case was phosphatidyl ethanolamine, giving 70 per cent of the total peak area of the phospholipid fraction. Minor components included diphosphatidyl glycerol (cardiolipin), 10 per cent of the total peak area, and ninhydrin positive material which migrated little from the base line. This latter material possibly corresponded to

Table 1. COMPARISON OF WHOLE CELL AND WALL COMPOSITION OF POLYMYXIN SENSITIVE AND RESISTANT STRAINS OF *P. aeruginosa*

	Wall as % of cell	REL as % of wall	Wall REL % PL % FN % EIF	Wall % PL % FN % EIF	Mg as % of wall	Mg as % of extracted wall	% of wall Mg extracted with REL	P as % of wall	P as % of extracted wall	% of Wall P extracted with REL	REL as % of cell	Cell REL % PL % FN % EIF	Cell % PL % FN % EIF	Mg as % of cell	Mg as % of extracted cell	P as % of cell	P as % of extracted cell
Sensitive																	
(a)	18.5	18.0	30	5.4	60	10.8	0.37	3.31	31	1.9	1.9	18	12.7	74.0	9.4	0.370	2.68
			10	1.8										24.0	3.1		2.5
			33.3	5.5										2.0	0.25		
(b)	18.2	16.5	66.6	11.0	0.37	0.31	30	1.9	1.9	16	—	—	—	—	—	—	—
			Nil	Nil													
			3.6	1.2										41.8	6.4		
Resistant																	
(a)	24.7	34.2	72.9	24.9	0.039	0.058	2.1	0.59	0.32	64	15.3	38.6	5.9	0.293	0.273	2.09	1.94
			23.5	8.0										19.6	3.0		
			4.7	1.3													
(b)	22.7	28.5	95.3	27.2	0.038	0.058	Nil	0.56	0.32	59	—	—	—	—	—	—	—
			Nil	Nil													

(a) Uncorrected values; (b) values corrected on assumption that ether insoluble fraction (EIF) is intracellular but sediments with same characteristic as wall fraction. (REL), Readily extractable lipid (chloroform/methanol soluble); (PL), phospholipid; (FN), free fatty acids and neutral lipids; (P), Phosphorus.

the phosphatidyl serine and lysophosphatidyl ethanolamine found by Bobo and Eagon¹⁵. Similar results were obtained with three other polymyxin sensitive strains of *P. aeruginosa*. These findings are in general agreement with those of Hancock and Meadow¹⁸ who concluded that the lipid content of the cell wall lipoprotein and of the cytoplasmic membrane was similar.

About 2 per cent of sensitive whole cell readily extractable lipid (chloroform : methanol soluble) was ether insoluble, but in resistant whole cells this was about 20 per cent. On the assumption that the ether insoluble material was a polymer of β -hydroxybutyric acid¹⁹ we attempted to hydrolyse it to crotonic acid with hot H_2SO_4 (ref. 20). Mixed melting points ($73^\circ C$) with authentic crotonic acid and the ultraviolet²⁰ and infrared²¹ spectra indicated that the original ether insoluble fraction was indeed a polymer of β -hydroxybutyric acid. Composition values in Table 1b were corrected on the assumption that the ether insoluble material occurred as intracellular granules but sedimented at the same centrifugal force as the wall fraction.

The phospholipid fractions from resistant whole cells and cell walls were greatly reduced in amount but were qualitatively similar to those of the sensitive strain; there was an indication that the proportion of phosphatidyl ethanolamine in the phospholipid fraction from the resistant cell walls was less than that in sensitive walls. The most striking difference between sensitive and resistant strains was in the cell walls. Resistant cell walls contained about 70 per cent more readily extractable lipid (excluding ether insoluble material) than sensitive cell walls. In spite of the overall increase in readily extractable lipid, resistant cell walls contained a quarter of the phospholipid and a tenth of the Mg^{2+} of sensitive cell walls. Although our results do not exclude the possibility of partial loss of constituents during wall preparation, the decreased Mg^{2+} and phospholipids in resistant walls is reflected in decreases in resistant whole cell preparations. With both kinds of cell, most of the wall Mg^{2+} was not extracted with the readily extractable lipid: the resistant walls lost a significantly smaller percentage of Mg^{2+} by extraction than did the sensitive walls. These results suggest that most of the wall Mg^{2+} , especially that in resistant walls, is not associated with the readily extractable lipid. The apparent increase in readily extractable lipid for resistant whole cells is accounted for by the increase in ether insoluble material. Similar reductions in wall phospholipid and magnesium were shown with cultures of *P. aeruginosa* NCTC 6750 trained to other levels of polymyxin resistance both in defined media and in nutrient broth.

The percentage of wall phosphorus extracted with the readily extractable lipid from resistant walls was much greater than for sensitive walls, even though the total phosphorus content of the resistant walls was lower. Thus for resistant walls there was a much smaller phosphorus content at sites not associated with readily extractable lipid, possibly implying a low lipopolysaccharide content.

Several workers have correlated cell readily extractable lipid with resistance to chemical antimicrobial agents²². Our results suggest that while the overall amount of readily extractable lipid (especially in the wall) is implicated in drug resistance, perhaps in a non-specific way, a decrease in the phospholipid fraction of the wall was a striking feature of the polymyxin resistant culture. This is in agreement with the low lipid phosphorus from walls of polymyxin resistant *P. aeruginosa* found by Newton¹¹.

We tentatively propose the specific involvement of phospholipid in the passage of polymyxin to the cytoplasmic membrane. In the presence of reduced amounts of phospholipid much higher concentrations of polymyxin are needed for it to reach its main site of action. The resistance to polymyxin of Mg limited cultures of an otherwise polymyxin sensitive strain of *P. aeruginosa*⁹ (the

same strain as used here, NCTC 6750) seems related to the drop in wall Mg in the present work. The role of wall magnesium may be directly or indirectly related to that of the phospholipid⁹. These results may be significant to the study of other membrane active drugs: they are compatible with Hamilton's hypothesis²³ that resistance to membrane active agents is due to the agent not penetrating through the wall. Polymers of β -hydroxybutyric acid commonly occur in sporulating organisms and may occur in the polymyxin resistant pseudomonad as an indirect consequence of the characteristics leading to resistance.

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Somatosensory Cortical Unit Responses of Long Duration

SINGLE cortical neurones have been reported to yield a short, high-frequency burst of action potentials in response to electrical stimulation of the contralateral body surface¹⁻⁴. We have found, however, that cortical neurones so excited usually respond with a long complex pattern of action potentials lasting from 140-2,000 ms.

Squirrel monkeys were anaesthetized by intraperitoneal injection of 65 mg/kg alpha-chloralose, and after introduction of tracheal and venous cannuli, they were paralysed by intravenous injection of 1 mg/kg 'Flaxedil'. The animals were artificially respiration and the pericentral cortex was surgically exposed. Bilateral pneumothorax was performed to reduce respiratory pulsation. Introduction of a microelectrode filled with 3 M KCl into the cortex allowed the action potentials of individual neurones to be recorded extracellularly. Recordings were taken from the precentral and postcentral areas (Fig. 1A) which have been reported to yield the shortest latency, largest amplitude primary evoked responses to contralateral forepaw (CFP) stimulation⁵, regions usually designated as the primary somatosensory cortex and primary motor cortex. The signals so obtained were displayed on an oscilloscope and were recorded on mag-

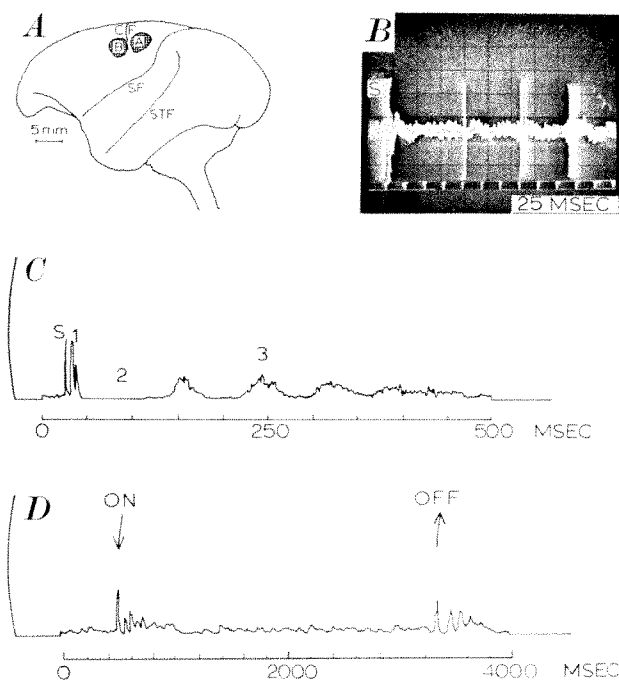


Fig. 1. *A*, Representation of the squirrel monkey cortex. *A*, Post-central and, *B*, precentral recording areas; CF, central fissure; SF, sylvian fissure; STF, superior temporal fissure. *B*, Oscilloscope record of the response evoked in a cortical unit by a 0.5 ms electrical stimulus to the CFP; S, stimulus artefact. *C*, PST of the unit response shown in *B*. 1, early excitatory phase; 2, inhibitory phase; 3, third phase of the response. *D*, PST of the same unit as in *B* and *C* evoked by a tap rather than an electrical stimulus. Arrows indicate the time of application and removal of the stimulus.

netic tape. The data were analysed on a Nuclear-Chicago Data Retrieval, or 'Line-8' computer ('Line-8' programs modified after W. Calvin)⁶. Stimulation was achieved by applying brief (0.5 ms) electrical pulses to bipolar needle electrodes inserted into the skin of the forepaw at a frequency of 1/5 s. Records were taken of 90 out of the 123 neurones examined.

A typical response evoked in a cortical unit by CFP stimulation is shown in Fig. 1*B*, and a post-stimulus time histogram (PST) of this response in Fig. 1*C*. The PST is generated from the data by computing the probability of the neurone under study firing an action potential in each of a series of time intervals following stimulation. Almost all the neurones we examined were spontaneously active so that a response is represented on the PST by a deviation of the probability of firing from the spontaneous level^{7,8}.

Responses were usually found to be composed of three distinct phases: (1) an early period of excitation, marked 1 in Fig. 1*C*; (2) a following period of inhibition, marked 2; and (3) a later generally excitatory period of variable form, marked 3. This three phase form of response was evoked by natural as well as electrical stimulation. Fig. 1*D* shows the PST response of the same unit as pictured in Fig. 1*C* when the forepaw skin was subjected to a tap rather than electrical stimulation. The tap produced a complex response with three phases at the time of application of stimulus ("on") and another three phase response at its removal ("off").

Typically, the first phase of the response consisted of a single high-frequency burst of 1 to 5 action potentials with a latency of 5–15 ms (mean 8.9) and a duration of 2 to 16 ms, recording precentrally, and a latency of 7–20 ms (mean 10.4) and a duration of 2 to 25 ms, recording postcentrally. This phase of the response is apparently identical with the short high-frequency burst of excitation evoked by CFP stimulation described in the literature for similarly situated cortical neurones.

Phase two of the response consisted of a period of time 200–500 ms (mean 200) in duration precentrally and 80–1,000 ms (mean 315) in duration postcentrally, during which the probability of neuronal firing was reduced below the spontaneous level. Phase two was present in about 80 per cent of the neurones examined, both pre and postcentrally. This phase may be the result of inhibitory postsynaptic potentials, because IPSPs of a similar duration have been reported to follow an initial excitation in the cortical neurones of the cat^{9,10}. The third and final phase of the response had a duration of 110–1,300 ms (mean 510) precentrally and 40–1,650 ms (mean 440) postcentrally. Phase three was present in 75 per cent of the precentral neurones examined and in 50 per cent of the postcentral neurones. In general this phase took one of two forms. (i) A series of bursts of action potentials with a well defined and reliable latency with respect to the stimulus. This form of the third phase of the response gave rise to a series of late oscillations in the PST as seen in Fig. 1*C*. (ii) A general increase in the frequency of firing, characterized by either single action potentials or bursts of action potentials which were variable in latency with respect to the stimulus. This pattern of response gave rise to a PST in which the third phase is represented by a smooth increase and decrease in the probability of neuronal firing (Fig. 2*A*). Forms intermediate between the two described were also observed. In addition another variation of the third phase of the response was sometimes seen (Fig. 2*B*) where the excitation, which usually comprised this phase, was followed by a distinct inhibition. (Note that because of the long duration of the PSTs shown in Fig. 2, phase one of the response has been compressed into the first few time intervals following the ordinate.)

The total duration of the three phase response was somewhat different pre- and postcentrally. Precentral neurones yielded a response lasting 140–1,700 ms (mean 735) while postcentral units yielded responses of 205–2,000 ms (mean 600) in duration.

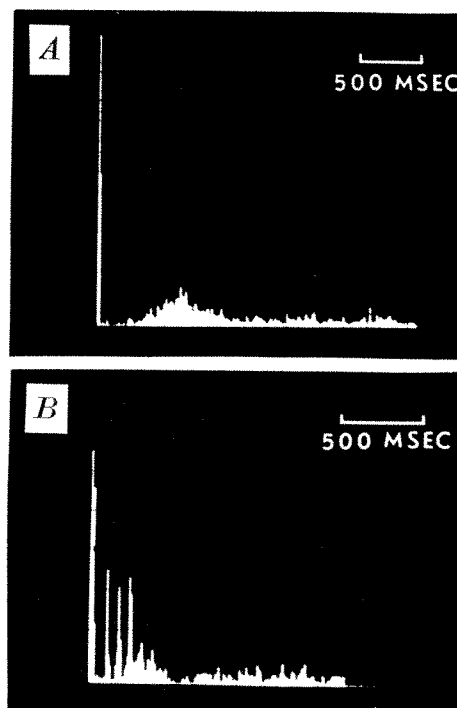


Fig. 2. PSTs of cortical units evoked by electrical stimulation of the CFP showing (*A*) a non-oscillatory third phase of the response and (*B*) an inhibition following the excitatory portion of the third phase of the response.

In addition to the complex three phase response described, some of the units which we examined yielded on stimulation only an early excitatory phase of 1-7 action potentials, with no discernible following inhibition and no late third phase response. This type of unit response represented about 20 per cent of our total population and is of the short, high frequency, burst type which has been previously reported as typical of primary somatosensory cortex¹⁻⁴.

The peripheral fields of the cortical cells were tested by the manual application of a nylon monofilament to the skin and in almost all cases the fields proved to be small and contralateral, but when the same units were observed under conditions of electrical stimulation of the skin it was found, in agreement with the observations of Towe *et al.* on the cat, that most of the units responded to ipsilateral as well as contralateral inputs^{10,11}. This was true of the neurones situated both pre- and post-centrally. The unit discharge pattern to ipsilateral forepaw (IFP) stimulation was also complex in nature, sometimes showing a three phase response similar to that described for CFP stimulation. We consider that the wider fields observed with electrical stimulation compared with those observed with natural stimulation may be attributable to greater synchrony of afferent input produced by a brief electrical pulse.

Our results indicate that the amount of information available at the cortical level in the form of the action potential patterns evoked by simple somatic stimuli is greater than had been previously realized. The patterns of discharge are at once longer and more complex, suggesting that neural coding of sensory information at the level of the primary sensorimotor cortex may involve processes more elaborate than hitherto suggested.

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X-linked Blood Groups, Xg, in Australian Aborigines and New Guineans

SINCE the discovery of the X-linked blood groups Xg by Mann *et al.*¹, the results of many important genetic studies on these unique blood groups have been reported. Race and Sanger² tabulated the Xg^a frequencies for different racial groups and noted that Chinese and Negroes have a lower Xg^a frequency than other peoples tested, and that Navajo Indians have the highest frequency, just slightly higher than that for Sardinians. In each

study reported, the blood samples had been collected from unrelated individuals.

The number of individuals tested are shown in parentheses, and the Xg^a frequencies reported were: North European extraction (5,388) 0.66; Sardinians (322) 0.76; Israelis, non-Ashkenazi (201) 0.68; Indians, Bombay (100) 0.65; Chinese, Singapore (64) 0.46; Chinese, mainland (171) 0.60; Chinese, Taiwan (178) 0.53; Chinese, Hakka (136) 0.53; Taiwan aborigines (164) 0.38; Mariana Islands, Chamorros (109) 0.65; Negroes, New York and Jamaica (219) 0.55; and Navajo Indians (308) 0.77.

Thus in the six years after the discovery of the Xg blood groups only nine different peoples were tested, and this, no doubt, was because of the need to conserve the original Xg^a antiserum for more important genetic studies. Through the generous gifts of anti-Xg^a serum in 1967 and 1968 from Miss Dorothy Henaman of the Hyland Laboratories, Los Angeles, and from Dr F. H. Allen, jun., of the New York Blood Center, New York, it has been possible to test progressively blood samples obtained from Australian aborigines of Central Australia, and New Guineans from the north coast and some central areas. In each of these series the blood samples came from men, women and children, and individuals in the separate Australian tribes and the New Guinea villages would be, in many instances, from related families. In this respect our two series differ from those previously reported. In a check made on some family results there were no exceptions to the expected Xg inheritance.

Table 1. Xg^a FREQUENCIES FOUND IN AUSTRALIAN ABORIGINES AND NEW GUINEANS IN A SAMPLE OF MEN, WOMEN AND CHILDREN*

Australian aborigines						
Total tested	Males	Xg(a +)	Xg(a -)	Females	Xg(a +)	Xg(a -)
352	163	139	24	189	175	14
		85 per cent	15 per cent		93 per cent	7 per cent
		$Xg^a = 0.79$		$Xg = 0.21$		
New Guineans						
Total tested	Males	Xg(a +)	Xg(a -)	Females	Xg(a +)	Xg(a -)
263	173	146	27	90	88	2
		84 per cent	16 per cent		98 per cent	2 per cent
		$Xg^a = 0.85$		$Xg = 0.15$		
		χ^2 for 1 d.f.				
				Males	Females	
				3.546	4.494	
				0.0015	0.012	
Australian aborigine						
New Guineans						

* The complete tribal and village details for each of these Xg studies will be published elsewhere.

The Xg findings for 352 Australian Aborigines and 263 New Guineans together with χ^2 for males and females in each series are presented in Table 1. The Xg^a frequency calculated for Australian Aborigines was 0.79, and for New Guineans was 0.85, and these frequencies are higher than any Xg^a frequency previously reported for other races of Man.

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Effects of Anaesthetics on Luminous Bacteria

LUMINOUS bacteria have been used extensively to study drug action¹, although studies of general anaesthetics in such systems have been limited to the effects of pressure on the inhibition of luminescence by ether and chloroform². The luminescent intensity, which is relatively easy

to measure, provides a direct indication of the effect of drugs on certain cellular processes. We believed that the use of luminous bacteria as a model system would offer an insight into the action of anaesthetics without many of the complications which occur in general anaesthesia of mammals.

Photobacterium phosphoreum, from the National Collection of Marine Bacteria, were supplied on an agar slant and were transferred to Oxoid nutrient broth (No. 2) containing 3 per cent (by weight) NaCl. Subcultures were grown at 21° C on a glycerol-salts medium based on that of Farghaly³.

Because the response of the bacteria to anaesthetics varies with the growth cycle, all samples for the luminescent intensity experiments were taken from cultures when the luminescence was at its maximum. This occurred in the latter half of the logarithmic growth phase and before the stationary growth phase. Although the absolute magnitude of the maximum luminescence decreases with successive subcultures⁴ there was no evidence that this affected the response of the luminescence to anaesthetics. Bacteria were centrifuged from the culture medium, resuspended in a phosphate buffer solution and continuously aerated in open glass sample cells at 21° C (the optimum temperature for luminescence). The luminescent intensity was measured with a photomultiplier tube (EMI 6094B) which had been calibrated with a standard light source. Dose response curves for the reversible inhibition of the bacterial luminescence by six general anaesthetics were determined. The bacterial samples were divided into two portions, one of which was used as a control and the other for the experiments. The volatile anaesthetics were admitted as vapour into the air supply which aerated the bacteria. During the experiments with the gaseous anaesthetics the bacteria were put in a sealed high pressure vessel with a 'Perspex' window, and stirred in the presence of sufficient oxygen tension to ensure stable luminescence.

The dose-response curve for ether is shown in Fig. 1. Luminescence is stimulated by low doses of ether and chloroform, and apparent stimulation was also observed for halothane and nitrous oxide but was not statistically significant. The dose-response curves were analysed in terms of a kinetic mechanism postulated by Johnson *et al.*⁵ in which the inhibitor is assumed to combine with an enzyme. This leads to a linear relationship between

$$\log \left(\frac{\text{per cent luminescence inhibited}}{\text{per cent luminescence uninhibited}} \right) \text{ and } \log P$$

(Figs 2 and 3) and this kinetic scheme provides a convenient method of handling the data. The partial pressures of the anaesthetics which reduced the luminescence by 50 per cent (ED_{50}) were determined from Figs 2 and 3 and are given in Table 1.

The relative oxygen consumptions of the bacteria were determined from the time taken for the luminescence to

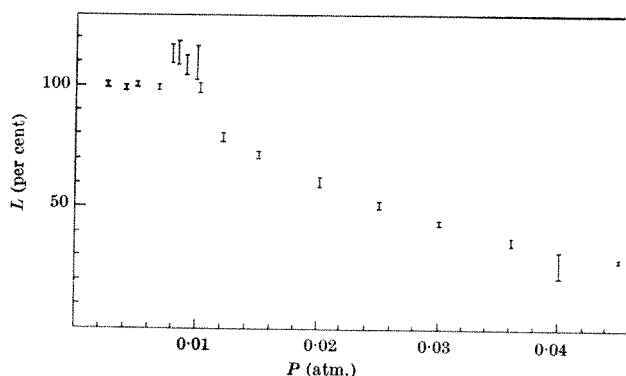


Fig. 1. Dose response curve, showing effect of ether on bacterial luminescence.

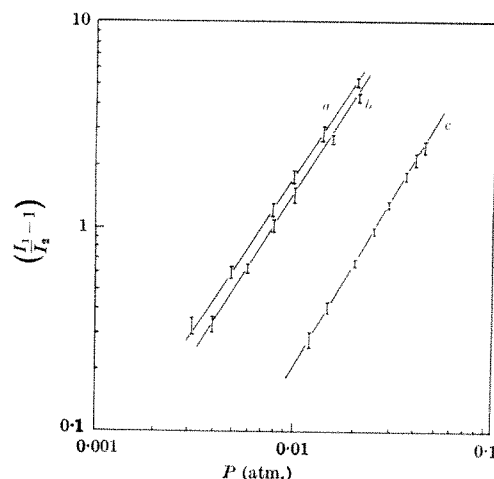


Fig. 2. Plot of $\log \left(\frac{I_1}{I_2} - 1 \right)$ against the log of the partial pressure of anaesthetic. I_1 and I_2 are the luminescent intensities in absence and in the presence of anaesthetic, respectively. a, Chloroform; b, halothane; c, ether.

dim after aeration of the solution had stopped, using the method of Harvey⁶ and Taylor⁷. It was found that, within the experimental error of ± 6 per cent, the presence of ether in concentrations which depressed the luminescence by 50 per cent of its original value did not change the total oxygen consumption. This can be interpreted as indicating either that luminescence and respiration are independent⁸ or that there are two pathways of electron transport that can support luminescence⁹. It does, however, seem unlikely that the total metabolism of the bacteria is being inhibited by the anaesthetic studied.

Table 1. COMPARISON OF THE EFFECTS OF ANAESTHETICS IN INHIBITING BACTERIAL LUMINESCENCE AND IN CAUSING GENERAL ANAESTHESIA IN MAMMALS

	Bacteria ED_{50} * (atmospheres)	Mice AD_{50} * (atmospheres)	Reference for AD_{50} s
Chloroform	0.007 ± 0.0001	0.008	13
Halothane	0.0081 ± 0.0001	0.0086	14
Ether	0.026 ± 0.002	0.032	13
$CHClF_2$	0.209 ± 0.004	0.16 ± 0.05	—
CCl_2F_2	0.50 ± 0.01	0.40 ± 0.06	13
N_2O	2.04 ± 0.05	1.50 ± 0.20	13

* The estimates of the errors of ED_{50} s are obtained from the standard deviation of the intercepts in Figs. 2 and 3, and those of the AD_{50} s are obtained from probit analyses.

We confirmed the observation of Eymers and Van Schouwenburg¹⁰ that 0.0003 M potassium cyanide will reduce respiration to 12 per cent and luminescence to 50 per cent of the levels observed in normal bacteria. It was found that in the presence of 0.0003 M potassium cyanide the ED_{50} s of ether and CF_2Cl_2 were not significantly changed (that of ether increased by 12 per cent and that of CF_2Cl_2 decreased by 5 per cent, which is within the error of these experiments in both cases).

When a suspension of luminous bacteria is allowed to dim through lack of oxygen and is then re-aerated, an "anaerobic flash" is observed¹¹. This is believed to result from a build-up of reduced luciferase which has been produced by reduced flavin mononucleotide¹². The results of the experiments on the effect of anaesthetics on the flash are summarized in Table 2. The maximum flash intensity is reached (to within 5 per cent) after 5 s of anaerobiosis. In the presence of ether, added before anaerobiosis, the flash intensity is reduced and there is no evidence that increasing the time of anaerobiosis in the presence of ether allows the flash to increase toward its original value. Finally, the addition of ether during anaerobiosis 10 s before re-aeration reduces the maximum flash intensity to the same extent.

The spectrum of the bacterial luminescence was observed in the presence of ether sufficient to reduce the lumin-

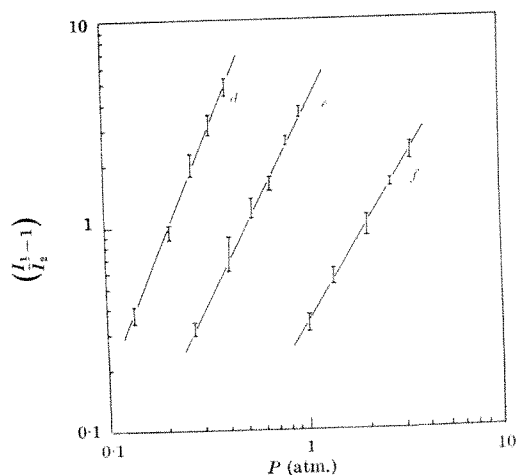


Fig. 3. Plot of $\log \left(\frac{I_1}{I_2} - 1 \right)$ against \log of the partial pressure of anaesthetic. I_1 and I_2 are luminescence intensities in absence and in presence of anaesthetic, respectively. d, CHClF_2 ; e, CCl_2F_2 ; f, N_2O .

escence to 50 per cent of its original value. The relative intensities of the luminescent spectrum and the wavelength of maximum emission (475 ± 3 nm) were the same as those of uninhibited bacteria.

Table 2. THE ANAEROBIC FLASH

Time of anaerobiosis (seconds)	Maximum flash intensity (arbitrary units)	Final steady state intensity (arbitrary units)
Controls		
5	7.2	4.3
10	7.3	4.3
15	7.45	4.3
20	7.5	4.3
Addition of ether (approx. 0.025 atm.) before anaerobiosis		
5	3.7	2.6
10	3.8	2.5
20	3.5	2.2
Addition of ether (in different concentrations) after 10 s of anaerobiosis		
20	4.0	3.2
20	3.7	2.5
20	3.6	2.2
20	2.1	1.5

The partial pressure of anaesthetic necessary to reduce the luminescent intensity by 50 per cent (ED_{50}) was compared with the partial pressure of anaesthetic which abolished the righting reflex of mice (AD_{50}) (see Table 1). The anaesthetic potency of monochlorodifluoromethane for mice was determined by a rolling response method which had previously been applied to other gases used in this work¹³. The anaesthetic potency used for halothane is that reported by Raventos¹⁴, and confirmed by Speden (personal communication—see also ref 15), rather than that published by Epstein *et al.*¹⁶. Table 1 shows that, for a wide range of anaesthetics, there is an excellent correlation between the anaesthetic potency in mammals and the potency observed in the inhibition of bacterial luminescence. The observed potency for chloroform, however, is markedly different from that reported by Johnson².

Previous workers have correlated anaesthetic potencies for mammals with the oil/gas partition coefficients^{17,18}, and with hydrate dissociation pressures at 0° C (refs 19 and 20). We have found that the correlation of anaesthetic potency for luminous bacteria with oil/gas partition coefficients was superior to that with hydrate dissociation pressures. This suggests, as in the case of mammalian studies, that the site of action of the anaesthetics is hydrophobic^{17,21}.

The further experiments which were carried out were intended to define more closely the nature of the hydrophobic region within the bacterial cell which was sensitive to the anaesthetics in concentrations which inhibited their luminescence by 50 per cent. Because the respiration is

unaffected by ether, it is unlikely that the total metabolism of the cell is being inhibited. The spectrum of the luminescence is unaltered and it thus seems unlikely that the light emitting complex is involved. The anaerobic flash experiments suggest that ether is not inhibiting the rate of formation of reduced luciferase. The simplest explanation of our results is that anaesthetics specifically and reversibly inhibit the luminescent system, possibly through their interactions with reduced luciferase. This interaction does not seem to be sensitive to the size or chemical structure of the anaesthetics except in so far as these could influence the solubility in a non-polar solvent. The remarkable parallel between anaesthetic potencies in mammals and in the inhibition of the luminescence system in bacteria suggests that the mechanism of general anaesthetics may be more specific than has frequently been supposed and may result from the interaction of anaesthetics with particular proteins within mammalian cells.

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Conflict between Positive and Negative Feedback as an Explanation for the Initiation of Aggregation in Slime Mould Amoebae

WHEN their supply of bacterial food becomes exhausted, some species of cellular slime mould seem to spread evenly over the area they occupy and later begin to aggregate into several fairly regularly spaced clusters. The aggregate typically migrates as a slug, then erects a fruiting body containing spores which germinate when conditions are favourable¹. What causes these seemingly independent organisms to engage in such collective behaviour? The answer to this question could provide important clues to the understanding of other morphogenetic developments.

Slime mould amoebae are attracted to a certain substance which they secrete themselves. (It is called acrasin, and has been identified in one species as cyclic-AMP².) The amoebae also secrete an enzyme (acrasinase) which

catalyses the breakdown of acrasin. We shall use these facts to explain the commencement of aggregation in terms of the interaction of like cells which each change in character during their life cycle. Details and a mathematical formulation will be published elsewhere³.

For our analysis the average amoeba behaviour can be most simply characterized by three parameters: (i) D_1 , a measure of chemotactic sensitivity which we define as the flux of amoebae per unit acrasin gradient; (ii) D_2 , an effective diffusion constant measuring the nonchemotactic random motion or motility of the cells; (iii) f , the rate of production of acrasin per unit mass of amoebae. We think that aggregation starts because of an instability of a uniform equilibrium state brought about by a change in these parameters. Another parameter necessary to characterize the system is K , an effective decay constant for the acrasin. Because K is a combination of the purely physical parameters characterizing the effects of acrasinase on acrasin it is not expected to change during aggregation.

The essence of the concept of instability is the assertion that a uniform distribution of amoebae and acrasin will never be observed unless the densities of the two inevitably return to their equilibrium values after being slightly disturbed. Disturbing perturbations may result from external influences, imperfect synchrony or the existence of special "initiator" cells detected by Sussman and his collaborators and ascribed to aneuploidy⁴.

Suppose that a relatively high concentration of amoebae and acrasin spontaneously arises in the vicinity of a point P . The factors important in determining whether or not this initial perturbation magnifies or decays are as follows. (a) The local concentration of amoebae tends to be dispersed by their random motion just as in ordinary diffusion. The magnitude of this effect is determined by the magnitude of the effective diffusion constant, D_2 . (b) Locally high concentrations of acrasin are dispersed by relatively rapid inactivation. K measures the magnitude of this effect. (c) The density of acrasin near P is increased because the amoebae concentrated there are producing acrasin at a rate f . (d) Additional amoebae are attracted to P by the local gradient in acrasin, thus increasing the amoeba density near P . D_1 is a measure of the magnitude of this response.

Factors (a) and (b) cause a negative feedback and tend to diminish a local concentration of acrasin and amoebae. Factors (c) and (d) cause a positive feedback and tend to magnify such a concentration. The uniform equilibrium state will be unstable if the net effect of the latter factors outweighs the net effect of the former. Mathematical analysis³ reveals that the critical balance occurs when $r \equiv D_1 f / D_2 K = 1$. A uniform distribution is stable when $r < 1$, and unstable when $r > 1$.

In terms of the present simple characterization of the behaviour of amoebae and acrasin, our hypothesis is that aggregation commences as a consequence of a change in one or more of the parameters D_1 , D_2 and f such that r increases from a value less than one to a value greater than one. Such changes in D_1 and f are known⁵ and have been quantitated⁶.

In our fuller analysis³, a mathematical model provides a precise form of the instability condition, now modified by the dependence of f on acrasin density, and an explicit formula for the decay coefficient K in terms of such quantities as the rate constants for the effects of acrasinase on acrasin and the equilibrium concentration of acrasinase. Incomplete analysis of our most detailed models indicates that the waves of fast inward movement, which are sometimes observed, are a consequence of instability which begins in an oscillatory manner. The density of fruiting bodies may also correlate with the perturbation wavelength which maximizes the instability parameter.

Analysis of our simplest model yields the qualitative prediction that aggregation in micro-drops will be delayed

and a quantitative prediction of the dependence of the parameter values at aggregation on drop area. If aggregation were primarily a consequence of the excessive production of acrasin by certain special cells, it would probably occur earlier in confined regions, for then the chemical message of special cells would be less attenuated and would reach the other cells earlier. Our analysis predicts that aggregation resulting from instability will occur later in confined regions, because perturbations in the concentration of acrasin have steeper gradients and are therefore more quickly vitiated by diffusion. This prediction has been borne out in one species⁷, which suggests that at least in this case it is correct to ascribe the primary role in aggregation to the change in average cell properties and a secondary role to the fluctuation in properties from cell to cell.

By formulating equations which describe the collective response of entities the motion of which is influenced by the concentration of an exterior factor, we have pointed the way to a theoretical analysis of other biological examples of symmetry breaking, such as those discussed by Platt⁸. It should be valuable to compare our detailed predictions for the slime mould with observation, particularly because the work reported here is, to our knowledge, the first to illustrate the stability concept on a well defined supramolecular biological system—the importance of which to developmental biology has recently been the subject of discussion⁹⁻¹¹.

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Separation of Effects of Auxin and Ethylene in Pea Roots

We have treated intact pea seedlings (*Pisum sativum* L. var. Alaska) with four synthetic auxins and found inhibition of primary root elongation, stimulation of radial swelling, proliferation of massed lateral roots and faster evolution of ethylene. Exposure to exogenous ethylene had the same effects, except that lateral root development was normal.

Many effects of auxin on higher plants seem to be mediated by auxin-stimulated ethylene evolution^{2-4,9,14}. Foliar abscission¹⁵, flowering in pineapple⁶, hypocotyl hook opening¹³ and petal wilt in orchids after pollination^{8,11} are believed to be mediated by ethylene. But the stimulation of the growth of stems⁷ and the herbicidal effects of some synthetic auxins are thought to be direct effects of auxin¹². We have investigated (1) whether synthetic auxins stimulate ethylene evolution in intact pea seedlings, and (2) whether pea seedlings exposed to exogenous ethylene develop abnormal roots as they do after exposure to synthetic auxins¹⁷.

Pea seedlings (48 h old) were exposed to four synthetic auxins: 5×10^{-5} M 2,4-dichlorophenoxyacetic acid (2,4-D), 1×10^{-5} M 4-amino-3,5,6-trichloropicolinic acid (picloram), 1×10^{-5} M 2-methoxy-3,6-dichlorobenzoic acid (dicamba) or 1×10^{-4} M 1-naphthalene acetic acid (NAA) in aerated solutions for 4 h. After treatment, seedlings were rinsed with distilled water and replanted in vermiculite.

The morphological response of seedlings treated with picloram, dicamba and NAA resembled the response of those treated with 2,4-D (Fig. 1). Longitudinal growth was inhibited immediately, and radial swelling developed 3 mm behind the root tips 12 h after treatment. Root primordia were initiated in the pericycle opposite the three xylem poles over the length of the roots 9 h after treatment, and massed lateral roots emerged about 72 h later (when seedlings were 120 h old).

Swelling of shoots after treatment with auxins has been attributed to ethylene evolution stimulated by the auxins⁷. We measured the evolution of ethylene and carbon dioxide in intact 52 h old pea seedlings. Respiratory CO_2 was trapped in potassium hydroxide, precipitated with barium chloride, dried and weighed. Ethylene evolution was measured by gas chromatography⁷ and the results were analysed with a DuPont curve resolver. The lower limit of ethylene detection was approximately 10 nl. g⁻¹ fresh weight h⁻¹.

Analysis of variance showed that picloram, NAA, dicamba and 2,4-D significantly stimulated the evolution of ethylene when compared with controls (5 per cent level) (Table 1). Only minor differences were found in the respiration rates among control seedlings and those treated with auxin, which suggested that the stimulated evolution of ethylene was not due to a change in the rate of respiration induced by auxin.

Etiolated 48 h old pea seedlings were exposed to a continuous flow of 50 p.p.m. of ethylene in air at a rate of 50 ml./min for 72 h in the dark, to determine whether exogenous ethylene could elicit the responses induced by auxin. Seedlings were grown upright with their roots through a stainless steel screen in a 3 l. glass chamber with a CO_2 trap. A layer of moist cotton in the bottom of the chamber prevented desiccation. After treatment with ethylene, seedlings were transplanted to moist vermiculite.

Extensive radial swelling and inhibition of elongation in both roots and shoots of seedlings treated with ethylene suggest that these responses are mediated by ethylene in seedlings treated with auxin (Fig. 1). Smith and Russell¹⁶ reported similar effects of ethylene on barley roots, but

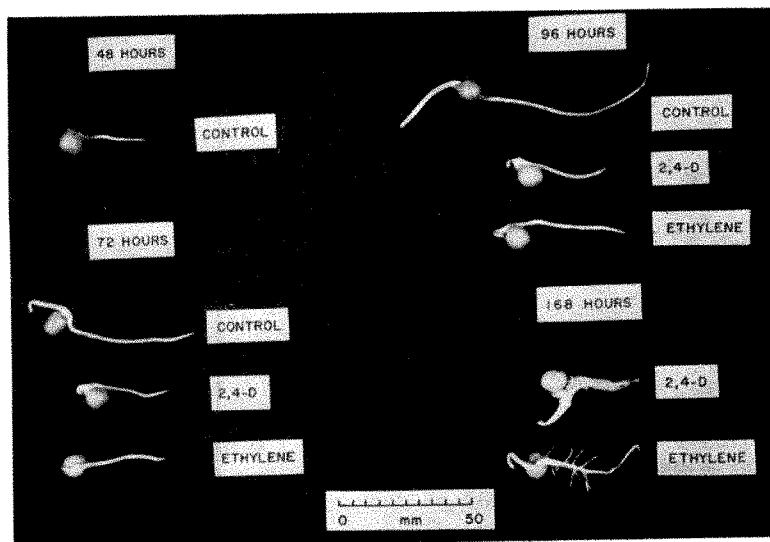


Fig. 1. Morphological development of etiolated pea seedlings exposed to 5×10^{-5} M, 2,4-D for 4 h or 50 p.p.m. of ethylene continuously from 48 h of age. The number of hours is the total seedling age when photographed.

Andreac *et al.*¹ disagreed. We did not observe the proliferation of massed lateral roots characteristic of treatment with auxin¹⁷. Lateral roots emerging from seedlings treated with ethylene seemed to be normal in size, number and distribution (Fig. 1). Ethylene, however, does stimulate the initiation of lateral roots in young stem tissue of many species¹⁰.

Was the concentration of endogenous ethylene in roots treated with auxin similar to that in those treated with ethylene? We consider that it was. Following the rationale of Burg and Burg^{4,5}, we calculate that the internal concentration of ethylene in the peas treated with auxin (Table 1) varied between 15 and 60 p.p.m., which agrees with the expected concentration of ethylene in seedlings exposed to a continuous stream of 50 p.p.m. of ethylene in air. We think that lateral cell divisions induced in the root pericycle by synthetic auxin are a direct effect on the course of cellular differentiation and are not mediated by the evolution of ethylene stimulated by auxin.

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Table 1. EVOLUTION OF ETHYLENE FROM ETIOLATED PEA SEEDLINGS

Treatment	Ethylene evolution (nl. g ⁻¹ h ⁻¹ ± 1 s.d.)
Control	26 ± 3.3
2,4-D	184 ± 5.5
Picloram	128 ± 29.9
Dicamba	51 ± 3.5
NAA	104 ± 10.4

Stabilization of Aphid Populations by an Aphid Induced Plant Factor

MANY tree dwelling aphids such as the sycamore aphid, *Drepanosiphum platanoides* (Schrk.), feed on the phloem sap of leaves and are restricted to one host plant. If therefore the aphids affect the metabolism of the leaves of deciduous trees while they are still developing in the spring, this may subsequently affect later generations of aphids.

In the course of a year the sycamore aphid typically shows three peaks of abundance, in spring, summer and autumn; and the spring and autumn peaks seem to be inversely related in size. The peak number of sycamore aphids in the autumn is low in years when the aphids are abundant while the leaves are still growing in the spring. High autumn peak numbers are achieved only in the years when few aphids are present in spring. The relationship between spring and autumn peak numbers of sycamore aphids recorded over a period of 9 years is expressed by equation (1):

$$A = S + 4.57 - 1.55S \quad (1)$$

where A and S are the logarithms of the autumn and spring peak numbers, respectively ($r = 0.92$, $n = 9$, $P < 0.001$, Fig. 1).

The quantity and quality of the amino-nitrogen present in the phloem sap of trees changes with the progress of growth and maturation of the leaves and shoots. When the leaves of sycamore are actively growing or senescing, as in the spring and autumn, the phloem sap contains relatively high concentrations of amino-nitrogen composed of many amino-acids; in the summer when growth has ceased the sap is poor in amino-nitrogen and it contains relatively few amino-acids. When the phloem sap is rich in amino-nitrogen and is a rich source of food the size and reproductive rate of the aphids both increase, and a large population of aphids develops. An abundance of aphids in spring, however, affects the nitrogen metabolism of the leaves, so that little nitrogen is translocated from the leaves before abscission, and the food available to aphids is then poor, so that few and small aphids with a low reproductive rate result^{1,2}. Thus high numbers of aphids in the spring can, through their effect on their host plant, adversely affect the number, size and reproductive rate of the aphids present in the autumn, two to three generations later.

In the autumn the parthenogenetic viviparous morphs of the aphid give birth to nymphs which develop into

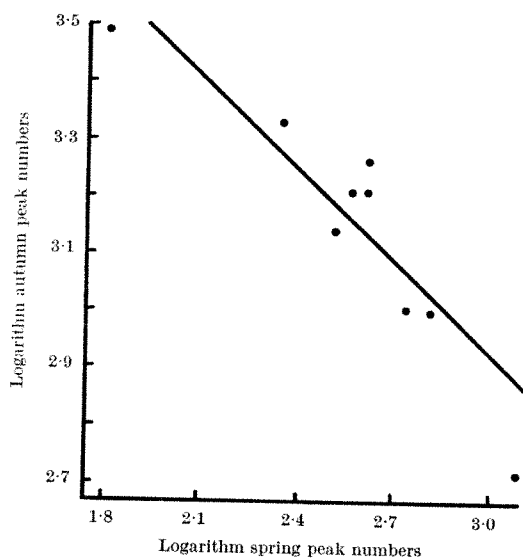


Fig. 1. Relationship between the spring and autumn peak numbers of aphids for each year from 1961-69.

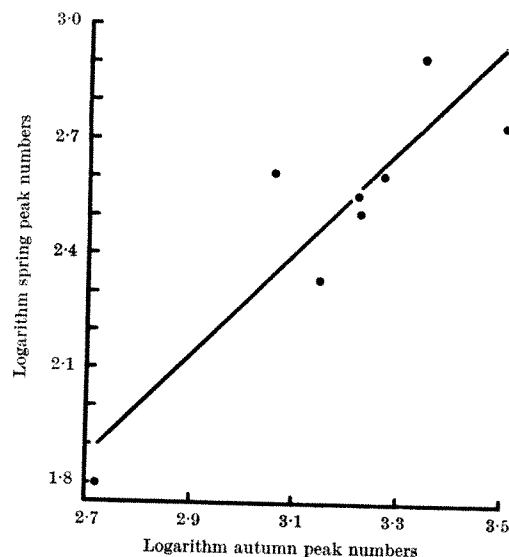


Fig. 2. Relationship between the autumn peak number of aphids and the spring peak number of aphids in the following year from 1961-69.

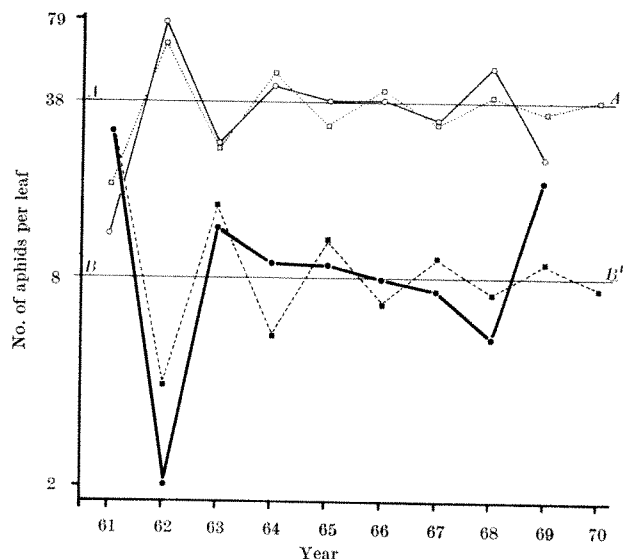


Fig. 3. Peak number of sycamore aphids per leaf in the spring (●—● observed, ■—■ expected) and autumn (○—○ observed, □—□ expected) from 1961-69. $A-A'$ and $B-B'$ = predicted equilibrium densities for autumn and spring, respectively.

males and oviparous females. The oviparous females lay the overwintering eggs in crevices in the bark of their host tree. The greater the number of aphids present in the autumn, the greater the number of sexual morphs that develop and hence the greater the number of eggs laid. The egg laying females are also larger and lay more eggs in years when aphid numbers are high. Thus proportionally more eggs are laid in years when aphid numbers in the autumn are high. The number of aphids present in the spring depends on the number of eggs that was laid the previous autumn and survives to hatch in the spring. The peak number of aphids present in the spring is related to the peak number present in the autumn of the previous year as shown in equation (2):

$$S = A - 1.6 + 0.3A \quad (2)$$

where S and A are the logarithms of the spring peak number in the current year and the autumn peak number of the previous year, respectively ($r = 0.88$, $n = 8$, $P < 0.01$, Fig. 2).

The changes from year to year in the spring and autumn numbers can be modelled using equations (1) and (2).

The aphid induced changes in nitrogen recovery from the leaves stabilize the population at eight aphids per leaf in the spring and thirty-eight aphids per leaf in the autumn (Fig. 3). Field observations over 9 years reveal that the peak numbers in the spring and autumn fluctuated from 1961 to 1963 and then came close to the equilibrium level from 1964 to 1967, and then fluctuated again from 1968 to 1969. Starting with the observed population in the spring of 1961 the model predicts fluctuations of decreasing amplitude taking 10 years to stabilize. The marked fluctuations in numbers from 1961 to 1963 and again from 1968 to 1969 were possibly the result in the first instance of weather factors, especially in the spring. In a late spring many of the aphids which are exposed on the smooth surface of the buds are washed off by heavy rain or eaten by birds before they gain access to the shelter of the unfurling leaves at bud-burst.

Aphid induced changes in sycamore leaves result in a delayed overcompensated negative feedback effect which stabilizes aphid numbers. By affecting the time of bud-burst, the weather could act as a density disturbing factor. Food plants may therefore play a more important part in the population dynamics of herbivorous animals than has hitherto been suspected.

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Equivocality of Survival of Learning acquired at the Larval Stage

Using larvae of the beetle *Tenebrio molitor*, Borsellino, Pierantoni, and Schieti-Cavazza¹ tested whether learning could survive through metamorphosis. Their apparatus was a maze consisting of a circular starting chamber, a short stem leading to a choice point and two alternative paths leading to a circular goal chamber. They describe training procedures in which "all animals passed through the maze once a day" and in which animals in the learning groups were punished for a wrong choice while larval controls were not. Punishment procedures exploited the strong negative phototaxis exhibited by both larvae and adults and consisted of a bright light introduced when subjects from a learning group entered the wrong path. After metamorphosis, adults of both the larval learning and control groups were passed daily, but without punishment, through similar mazes. The results indicated that controls performed at chance levels while learning subjects significantly chose the side they were trained to enter earlier as larva. If their conclusion "that learning survives through metamorphosis" were justified, if their results were convincing, and if their procedures were acceptable, this study would indeed be very exciting.

But did learning occur in larval animals? Did learning survive through metamorphosis? Critical examination of this study indicates that both questions can only receive equivocal answers, for the data generated by their procedures cannot be interpreted unambiguously.

First the results themselves are not convincing. Certainly the probability values reported are significant and large. Nevertheless, converting their data to percentages indicates that the effect is very small. Larvae in the learning conditions show approximately 60 per

cent correct responding during training as larvae and later during testing as adults. Such a level of responding on a learning task would be relatively low even when compared to a baseline of zero. But in the present two choice situation the baseline is much higher and an increase from a chance level of 50 per cent correct responding to the 60 per cent obtained suggests an inherently weak effect which, while consistent with a learning interpretation, could just as easily and reasonably be attributed to an unnoticed source of bias.

Other potentially useful data were unfortunately not reported. By collapsing data into a single set of scores, as they did for larval training and adult performance, effects can be obscured or eliminated. In an investigation of learning this is particularly important, for systematic changes in performance would be expected with additional training. What was the course of correct responding over larval training and adult performance trials? Was there a gradual improvement in responding during larval training? Then too, because punishment was withheld during adult performance, the method of testing at this stage was essentially an extinction procedure. Considering that the 62 per cent level of correct responding for adults was almost identical to that obtained during larval learning conditions, why then was there no evidence for extinction? Or was there a gradual reduction in responding during adult testing? But even if it could be shown that in every animal training results reflect initial chance responding followed by improvement to a typical learning criterion, for example, an eventual run of nine out of ten correct choices, while adult performance reflected the characteristic reduction of responding associated with extinction, this experiment would still be unsatisfactory.

A related point concerns the post-metamorphosis comparison between larval learning and control adults. During this stage both groups were passed through mazes without punishment and on average 17 days of metamorphosis occurred between the end of larval training and adult testing. It is at least suspicious that as adults there is no evidence of forgetting at all. If learning occurred in the larvae, one should minimally recognize the potential for forgetting to occur as well, effects which would make the recall test used (without punishment) insensitive and unsatisfactory. In the absence of precise empirical evidence for larval memory, the prudent and quite possibly more appropriate comparison, anyway, would compare relearning scores for the larval learning group with original learning scores for a comparable adult group.

But perhaps the most puzzling problem is why these animals should have responded or learned at all. Investigations of animal learning usually include motivational components which activate and direct behaviour to relevant goals. Identifying light as the punishment or negative reinforcer relevant for the characteristic negative phototaxis of *Tenebrio* will not do because it will only account for the organism's selection of the correct path. A basic problem of motivation remains unanswered. Why do these beetles (larval or adult; trained or untrained) ever leave the start box and initiate a trial? In this experiment the start box is presumably just as consistently a safe (and dark) place as the goal box, for the relevant motivation was never introduced until the choice point when punishment procedures were introduced for wrong responses.

Perhaps the rate of activity in *Tenebrio* is sufficiently high that the animals would spontaneously leave the start box and approach the choice point. Assuming that *Tenebrio* is capable of learning, there is yet another question: why did they learn to make the correct turn rather than learn to remain passively in the start box?

Quite possibly all of this could be quickly resolved if we knew what specific procedures are implied by "passing an animal through a maze". As it stands this is surely vague and ambiguous and fails to communicate a single

replicable feature of their procedures. It has been suggested to me that perhaps individual animals were forced, by jabbing, pushing and so on, to initiate a trial and traverse the maze. But such inelegant procedures, while often standard practice with invertebrates, are themselves objectionable, for they may introduce systematic bias by permitting the experimenter unintentionally to guide an animal's movements². Such a bias could easily account for the small effect reported.

I thank the Department of Zoology, University of Edinburgh, and Dr A. Manning for the use of facilities while preparing this manuscript.

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Received March 19; revised June 15, 1970.

¹ Borsellino, A., Pierantoni, R., and Schieti-Cavazza, B., *Nature*, **225**, 963 (1970).

² Ratner, S. C., *Psychol. Rec.*, **14**, 31 (1964).

Reply to Comment on Survival of Learning acquired at the Larval Stage

REYNIERSE'S criticism¹ of our work on the survival of learning acquired at the larval stage raises doubts about whether learning does occur at all in larval animals.

Disregarding the long-standing discussion about the exact nature of learning (for example, ref. 3), our criterion for maze learning should satisfy the requirements mentioned by Reynierse. Considering only the results of the first trial of each day, we take for the k^{th} day a statistical variable x_k , with values ± 1 ; $+1$ for a correct choice, -1 for a wrong choice. We plot the total displacement

$$X_N = \sum_{k=1}^N x_k$$

due to the accumulation of ± 1 steps until N^{th} day. A few typical examples of such runs are shown in Fig. 1. These data show that after an initial period of chance responses, there are increasingly long runs of correct choices.

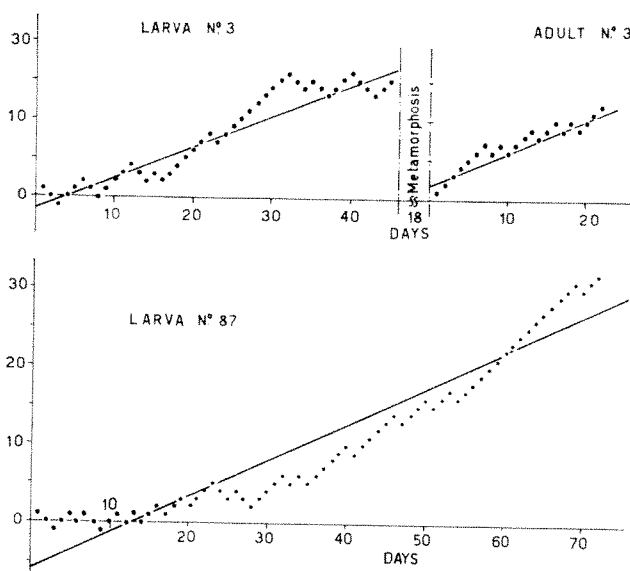


Fig. 1. Two examples of runs. The upper one shows also the adult performance after metamorphosis. The ordinate shows the cumulative sum of daily steps $+1$ (correct choice), or -1 (wrong). The slope of the fitted straight line i is a measure of the general trend.

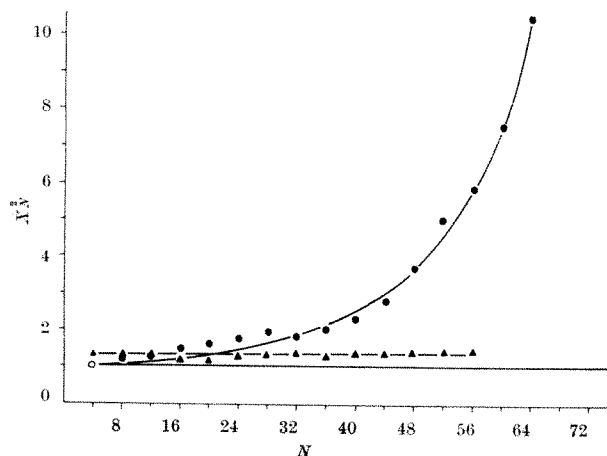


Fig. 2. Ratio of the variance \bar{X}_N^2 , taken over the population, to the expected value N for random behaviour. See also text. ●, Learning group; ▲, control group.

We fitted the 200 individual diagrams with straight lines—using the least square criterion—to obtain a parameter measuring their trends (Fig. 1). While the distribution of the slopes of such lines for the control group is symmetrical around slope zero, within statistical fluctuation (positive/negative = 48/44), the distribution for the trained group is definitely asymmetrical (positive/negative = 62/38).

The cumulative variable X_N , when averaged over the population for random choices, gives $\bar{X}_N = 0$; its average square instead has $\bar{X}_N^2 = N$. So the ratio \bar{X}_N^2/N ought to be constant and equal to 1 for a population of individuals making completely random choices. Although this ratio is constant (and equals 1.2) for the control group, for the trained larvae it increases regularly with time (Fig. 2). The performance of the trained population improved continuously.

We believe that we have answered the questions raised about the ability of larvae of *Tenebrio molitor* learning in a maze. As for their motivations in so doing, we have nothing new to add to the "explanations" or hypotheses that are already well known² and used of the many theories advanced so far.

We were very careful in planning and in executing our experiments, which were started in September 1964, repeated in better controlled conditions in 1966 and reported as a preliminary note in 1967⁴. We believe that these results indicate that the animals exhibited learning behaviour rather than the experimenters.

After we had written this reply, we received a reprint⁵ which described the training of *Tenebrio molitor* larvae followed by testing for the persistence of memory traces after metamorphosis. Apart from small differences in detail, the conclusions were the same as ours.

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¹ Reynierse, J. H., *Nature*, **227**, 1369 (1970) (preceding article).

² Borsellino, A., Pierantoni, R., and Schieti-Cavazza, B., *Nature*, **225**, 963 (1970).

³ Hindle, R. A., *Animal Behaviour*, 568 (New York, 1970).

⁴ Borsellino, A., Pierantoni, R., and Schieti-Cavazza, B., *Atti Cong. Gruppo Naz. di Cibernetica*, Pisa, 20–21, 29 (1967).

⁵ Cherkashin, A. N., Shermain, I. M., and Stajekhina, V. S., *Akad. Nauk. USSR Physiol.*, special issue, 117 (1968).

Book Reviews

STUDENTS IN BRITAIN

The Rise of the Student Estate in Britain

By Eric Ashby and Mary Anderson. Pp. x + 186. (Macmillan: London, September 1970.) 50s boards; 20s paper.

"Who do they think they are?" About a year ago a more colourful version of this rhetorical question was put to me by an angry middle-aged Derbyshire miner who was much troubled by what he regarded as the lunatic activities of some university students. He was especially indignant at their apparent misuse of educational opportunities which he had wanted for himself twenty years ago but which he had been denied. Particularly galling to him was the thought that his money was being spent on university education when capital expenditure on the primary and secondary schools of his area was desperately needed, and he urged me to throw these wastrels and mischief-makers out of the university. I imagine all vice-chancellors and academics can recall similar experiences and have faltered for words to explain that universities are places where reasonable dissent must be not merely tolerated but encouraged, that it is inherently desirable that there should be responsible student leaders but that these can often only remain in office and repel the extremists by stealing some of the latter's fire, and that current student unrest should be seen in its historical perspective. Had we read Ashby and Anderson's book, how much better might our answers have been.

The authors trace the growth of the student movement in Britain over the past 150 years. It is, no doubt, historically accurate. I am no judge of this, but my own close contact with students in Oxbridge and civic universities convinces me that even if professional historians should subsequently find fault with some of the facts the interpretations of the events recorded in this chronicle are all fundamentally sound. Every page makes compelling reading, and there are many treasures in the form of bizarre facts or wise and perceptive judgments. Who would think that the notion of a self-governing hall was current and acted on in Edinburgh 83 years ago, or would consider it likely that the predominance of medical students once made for a lively and active student body, or that the National Union of Students owed much more in its early days not only to its first president (Macadam), but also to those pillars of the establishment of the 1920s, such as Nancy Astor, Lord Haldane and the editors of *The Times* and *The Observer*? Also interesting and slightly surprising is the fact that many current issues concerning the status and role of students in universities were initially raised many years ago only to be dropped and either resuscitated or even rediscovered several years later. Quite frequently distinguished men and women unconnected with the organized student movement have been more cogent and passionate advocates of reform of the curriculum, of pedagogic method, of examinations and university structure than the students.

The final chapter called "The Conscience of the Student Estate" is of outstanding interest. It contains a brilliant analysis of the elements of the student body: identifying

the dedicated wreckers uninterested in reform and devoid of hope or humour who would smash their own student union as willingly as their university, and whose tortured minds are nourished by absurd slogans such as "creative vandalism" and "repressive tolerance". It is full of sound advice to administrators on how to de-escalate situations deliberately contrived to lead to confrontation; it spells out why "open democracy" can never work, and how power in the university is so widely diffused that to seek the source of power is to chase a will-o'-the-wisp. The knife edge trodden by academics between hated paternalism, on the one hand, and equally disliked indifference, on the other, is made plain. The NUS receives generous tribute for its common sense and responsibility, but is brought face to face with the stark alternatives; either that students are to be regarded as part of the community of the university and eligible for representation on committees, in which case their union, like any other constituent official body of the university, must be regulated by the university; or the union is like a trade union claiming autonomy and negotiating rights, in which case student membership of university committees is ludicrously inappropriate.

There is singularly little evasion of issues but perhaps the most important question of all is left open, namely, whether the growth of the student estate has led to any substantial improvement of the education of 18-21 year olds which might otherwise not have occurred. In so far as the single most influential factor in education is the attitude of a teacher towards his pupils, and in particular his feeling of commitment to them, it seems doubtful to me that the organized student movement has made any real gains for the student. It could even be argued that in the past two years as the direct result of student activities there has been a decline in morale and staff concern for students which will take a long time to repair. Meanwhile, this book should be compulsory reading for all dons and students.

F. S. DAINTON

MEDICS THROUGH THE AGES

The History of Medical Education

An International Symposium held February 5-9, 1968. By C. D. O'Malley. (UCLA Forum in Medical Sciences No. 12.) Pp. xii + 548. (University of California: Berkeley, Los Angeles and London, August 1970.) 190s.

In February 1968 a symposium on the history of medical education, organized by the late Professor C. D. O'Malley, was held at the University of California at Los Angeles. The papers, which were read by nineteen international experts, have now been collected together with illustrations and an index which includes far more individuals' names than subject titles. The articles are arranged in four groups, the first two being chronological periods and the remainder geographical. The opening section, "The Earlier Period in the West", deals with classical antiquity, medieval Islam, the middle ages, and the renaissance. "The Modern Period" includes essays on medical education in the major European countries, and "Eastern Europe and the Far East" covers Russia, India, south-east Asia, and Japan. Finally, "Western Hemisphere" is concerned with Iberia and Ibero-America, and with the United States before 1900, and in the late nineteenth and twentieth centuries. Each paper is the work of an outstanding scholar and they are all of high quality, being well written and impeccably documented. There is, however, some variation in size, for, whereas France occupies 52 pages, Ibero-America 44 and classical antiquity, India and Scandinavia 37 pages each, Scotland has 11, England and Italy 14½ each, the Netherlands 15½ and Germany 28. There is therefore some degree of disproportionate representation, as measured by importance and overall contribution. In the case of Austria,

only one topic, the evolution of clinical teaching in Vienna, is discussed.

For a variety of reasons, interest in medical education today is universal and significant reforms are being considered and introduced all over the world. But in order to appreciate fully the present situation, it is usually necessary to trace out their complex origins, proceeding from the simple to the complicated. Only by doing this can a full understanding of present day relationships, pressures, influences and of formative factors be achieved. Moreover, although conditions vary considerably from country to country, certain basic necessities are common, and the manner in which a problem presents and is tackled in one country may have relevance to reformers in another. The editor, recognizing the need medical educators have for an historical approach, has provided a book from which they will benefit greatly. As he points out, the only comparable treatise was published almost 80 years ago, by Theodor Puschmann, and has little interest for modern readers who are not concerned with history of medicine *per se*.

This book, however, not only brings the story up to modern times but also includes material which cannot be readily found elsewhere, and certainly not in English; thus, although the main trends and developments in many European countries are widely known, events in Asian countries have until now been little appreciated in the West. But Professor O'Malley realized that inevitably there would be many lacunae and it is hoped that his presentation will stimulate historians to fill them. In the case of Britain, for example, much has been written about the general developments, the metropolitan institutions and their conflicts, and about legal reform of the profession and its effects on medical education. We need in addition, however, much research into neglected areas: studies of the curricula and library and laboratory facilities as they reflect the diffusion of science into medicine; details of provincial training; the actual numbers of students, trainees, graduates, and the like; and on the whole a broader approach to many problems, based on the social, general historical, and economic elements rather than on medical issues and carried out by adequately experienced historians rather than by medical men.

In several ways, therefore, this book is an important contribution to the history of medicine and in particular it is informative and inspirational. It is also one of the last publications of Professor O'Malley, whose enthusiasm, erudition, and guidance in the field of medical history in general will be greatly missed in the future.

EDWIN CLARKE

KING AMONG THE BLIND

Visual Perception

By Tom N. Cornsweet. Pp. xiii + 475. (Academic: New York and London, May 1970.) 140s.

EVEN if there were room for argument as regards the readability of this beautifully produced book, there cannot be any about its legibility. The type is excellent. The illustrations (by Peter Howland) are beyond praise, and some have the smoothness of a lithograph. It is only in a state the soil of which had been trodden by the ardent emissaries of the Conquistadores, such as California, that a sardonic monocular skull could have been produced to illustrate the oculomotor system. Did I say monocular? It seems hardly credible that one of the most sophisticated of visual perceptions—namely fused binocular vision to yield stereopsis—failed to get lodged in these extensive and informative pages, which modestly claim to include “the fundamental topics underlying the entire, broad field of visual perception”. True,

fusion is taken for granted in a system which, the author seems to believe, could convert monochromats into dichromats, and dichromats into trichromats. But otherwise we are one-eyed.

Having stressed what the book omits, I have to tell you what it covers. About 20 per cent of the book deal with threshold phenomena with special reference to the study of Hecht, Schlaer [sic] and Pirenne. Another 14 per cent goes into a little photochemistry (with yet another magnificent artist's impression of molecular disruption) a trifle of receptor physiology, a wee bit of dark adaptation a speck on spectral sensitivity, and a rather nice soupçon of Stiles-Crawford. The next 24 per cent enters into colour vision. The rest of the book deals essentially with spatial and temporal interaction, being illustrated by well thought out experiments, clever and also beautiful photographs, and it shows that Professor Cornsweet not only has this particular subject at his fingers' tips but also knows how to present it. This is really well worth reading.

The student will wish to concentrate on this section. The earlier parts give the impression that the author absorbs wisdom more readily from loud writers than from deep ones. His photochemistry is insufficient to prove to him the fallacy of Fig. 9.2b. And nowhere does he acknowledge the debt Figs. 8.12 *et seq.* owe to Willmer's pioneering thoughts. One would have liked to see some mention of small-field tritanopia—on which the evidence is good—and much more on tetrachromacy. Page 151 contains several factual errors, and I have read the original in vain to find where Purkinje states that he “passed white light through a prism and looked at the resulting spectrum during the early and the late stages of dark-adaptation . . .”.

The final section is, however, well integrated, and the psychology of perception warmly shakes comparative biology by the hand. It contains a very good account of the theory and practice of modulation transfer functions and Cornsweet has spared no effort to produce one of the best introductions to the ins and outs of brightness that has yet been written. If the student finds the underlying physiology simplified, he should be grateful, and this, incidentally, is a feeling which many of us will share when we sense our several consciousnesses joggled by ideas they had not previously harboured.

R. A. WEALE

A COMPLETE LABORATORY

Biochemistry of the Eye

Edited by Clive N. Graymore. Pp. xiii + 783. (Academic: London and New York, June 1970.) 240s; \$12.

As Sir Stewart Duke Elder says of the eye in his foreword in this book, “This small and readily accessible organ contains in its restricted compass almost every type of metabolic reaction. . . . It is indeed a complete biochemical laboratory”, and one is grateful to Dr Graymore and his team of contributors for collecting the basic information and the later advances together in one volume. The book contains chapters on the cornea (Maurice and Riley), the aqueous humour and ciliary body (Cole), the vitreous body (Berman and Voaden), biochemistry of vision (Bridges), biochemistry of the retina (Graymore), inborn errors of metabolism affecting the eye (Graymore and Hsia), nutritional aspects of the eye (McLaren), and three chapters on the lens (Kuck). All the chapters are informative and well documented. The biochemistry of the separate parts of the eye is as different as their embryology and functions, and biochemists who are not already working with eye tissues may well find on reading this book that some part of the eye—the lens, the retina, the vitreous body—could provide a biochemical system that would help solve their problem.

The chapters on the lens contain much useful information but I feel that too much space is given to cataloguing constituents of the lens. Some rather odd statements are made, such as (p.184): "True cataract is a conversion of the normally transparent lens protein to a coagulated and opaque state, not unlike the white of a hard boiled egg". This is untenable. Near half the cataracts removed at operation have no more insoluble protein than non-cataractous lenses from people of the same age.

The chapter on the biochemistry of vision by Bridges is one of the most valuable in the book and includes discussions of retinaldehyde-opsin bonds in the visual pigments, the structural changes in rhodopsin associated with bleaching, the photoproducts formed and their reactions in the eye. Graymore gives a spirited account of our imperfect knowledge of the general metabolism of the retina and discusses changes that take place when it degenerates either as a result of chemical insults or in hereditary diseases of animals which may be similar to retinitis pigmentosa of man. We need to know so much more of retinal metabolism to understand retinal disease that one hopes this book will stimulate an explosive outburst of biochemical work.

This book is to be welcomed, but it seems sad that Academic Press should produce two books, whose contents and writers overlap, on this specialized subject within the space of one year. The second edition of volume one of *The Eye* (Davson) appeared in 1969, and now we have this *Biochemistry of the Eye*. This may be a sign of vitality, but it would have been better for the subject if these books had been spaced.

ANTOINETTE PIRIE

NORTH AMERICAN DIPTERA

The Flies of Western North America

By Frank R. Cole, written in collaboration with Evert I. Schlinger. Pp. xi + 693. (University of California: Berkeley, Los Angeles, and London. July 1970.) 238s.

ONE of the most difficult tasks confronting an author when writing a large general work of this nature is to strike a balance between providing a detailed taxonomic treatise, of rather narrow appeal, and what is commonly called the popular approach. The author, F. R. Cole, and his many associates have succeeded in producing a work which should be of value not only to dipterists but also to biologists in many related fields.

This work is an attempt to consider, in far greater detail than is available at present, all the two-winged flies of western North America: that is to say, most of that region west of the 100th meridian, including Alaska and the southern California peninsula. C. H. Curran's *The Families and Genera of North American Diptera* is used as a basis and this may automatically devalue this work in the eyes of many dipterists. Such, however, has been the revision that there is only a limited similarity to Curran's pioneering effort.

It is impossible in a work of this magnitude and in a discipline as subjective as taxonomy to expect that the author will not receive a considerable amount of criticism. Much of this criticism will be trivial in nature, but unfortunately it is at this level that this work is particularly vulnerable. In an attempt to enliven and vary the introductions to families and genera certain words and phrases are used, for example, "off-beat", "way out", and the like, which may well cause either amusement or irritation.

There is a substantial introduction, a major part of which is a description of the region under review, written in a travelogue style, successfully combining a great deal of geological, biogeographical and strictly biological information. The section on general anatomy, based on Crampton, covers the subject in detail to a degree out

of keeping with the remainder of the book; much of the discussion could probably be omitted without seriously affecting its value.

The principal part of the book concerns the taxonomic treatment, with keys to genera and family groups, based for the most part on the most recent works available. A list of the species known to occur or reported from the region is included under each genus. Bionomic data, usually of a distributional nature, are also given for each species, although occasionally sufficient anatomical detail is also provided to enable species to be determined. Line drawings, more than 400, of a very high standard are used throughout to good effect; some are reproduced from the Curran manual and other sources, but the majority are original and executed by the author. There are inexplicable lapses, including a few rather poor drawings, some of which have been apparently labelled free-hand, but these only serve to accentuate the high quality of the remainder. A full index and glossary, together with a more than adequate selected bibliography of more than 1,600 items, round off this most useful work.

B. H. COGAN

PHYTOPHAGOUS INSECTS

Insect and Host Plant

Proceedings of the Second International Symposium, Wageningen, The Netherlands, June 2-5, 1969. Edited by J. de Wilde and L. M. Schoonhoven. (Reprint from *Entomologia Experimentalis et Applicata*, Vol 12.) (North Holland: Amsterdam and London, 1970.) Hfl. 54; 126s; \$15.

THIS is a valuable summary of current theories and recent research on the finding and utilization of food plants by insects. Most of the twenty-eight papers are of a high standard, but it is to be regretted that there is no record of the discussions which must have followed their presentation.

Twelve papers chiefly deal with the recognition and location of the host plant by the insect. There are papers by Fraenkel on secondary plant substances, Kennedy and Moorhouse on locust responses to wind-borne grass odours and Moericke on host-specific colour behaviour by an aphid; but most of this section is devoted to electrophysiological investigations of chemotactic receptors and the effects of these receptors on feeding behaviour. Dethier and Schoonhoven have an ingenious explanation of how these could be used for olfactory coding.

Five papers are concerned with nutrition, particularly artificial diets. Work on aphids is reviewed by Auelair. House shows that most phytophagous insects have similar qualitative food requirements, but that the proportion of each nutrient in a diet contributes more to nutritional quality than the absolute amount. A further five papers are concerned with the effects of food plants on growth and metamorphosis, the review by Sláma of insect hormone analogues occurring in plants being of particular interest. Miles gives a lucid account of the probable role of phenols in relations between plants and Hemiptera. The remaining papers are concerned with various aspects of plant resistance to insect attack and include descriptions of large-scale breeding programmes for resistance in rice by Pathak and in cotton by Maxwell and his colleagues. In this section, as in the whole symposium, the resistance phenomena termed "preference" and "antibiosis" by Painter receive much attention. There is very little discussion of the third aspect of the resistance of plants to insect attack, which Painter called "tolerance" and which is concerned with the growth of attacked plants. Perhaps this very important aspect of insect/plant relations will receive greater attention at future Wageningen symposia.

R. BARDNER

MODEL ECOLOGY

The Ecosystem Concept in Natural Resources Management

Edited by George M. Van Dyne. Pp. xii + 383. (Academic: London and New York, January 1970.) 154s.

"An ecosystem results from the integration of all of the living and non-living factors of the environment for a defined segment of space and time." It includes the plants, animals, soil and associated microclimate. Clearly it is a complex system and ecologists are only now learning to describe ecosystems, both in terms of their structure and composition and of their functioning. Utopia lies ahead; if we could understand how ecosystems function and could make not only descriptive models but predictive ones, we could use this concept in managing them. That is, we could apply it to the efficient management of forests, parks, nature reserves, recreational areas, water catchment areas, lakes and rivers, sea fisheries, deer forests, prairies and so on. The models would be based on systems analysis which is a form of magic designed to cover up the uncertainties in our measurements and interpretations and still allow the computer to come up with the (right) answer. Some of us, being a little uncertain of the appropriate incantations to achieve this magic, were hoping to find the answer in this book.

But, in fact, systems analysis is logic, not magic. In a sense it is nothing new, just an organized way of studying the elements of the ecosystem, their states and their relationships with one another. Of course the total complexity of the system must be simplified into a model and the model can be no more precise than the observations and hypotheses on which it is based. The first agricultural author (possibly Virgil) to note that cow eats grass was deriving a simple verbal model from observation. It can also be formulated as



or more precisely as

$$\begin{array}{l} \text{intake of grass} \\ \text{by cows} \end{array} = \begin{array}{l} \text{faeces + urine} \\ \text{produced by} \\ \text{cows} \end{array} + \begin{array}{l} \text{growth, repro-} \\ \text{duction and} \\ \text{milk produced} \\ \text{by cows} \end{array} + \begin{array}{l} \text{respiration} \\ \text{by cows} \end{array}$$

all in some suitable unit such as watts/m²/day.

But it is only in the past ten years that this approach has been used to tackle the complexity of whole ecosystems. It needed ecologists with the confidence to use computers. It needed also some large multidisciplinary studies of ecosystems such as have been especially promoted by the International Biological Programme. This book provides a good review of the present state of progress.

The thirteen authors involved approach different ecosystem problems in different ways. There is no simple or universal recipe for successful modelling. Some chapters, like that on the Canadian IBP prairie project at Matador, describe the approach to be used in a study still very much in progress. Other chapters, such as that by Schultz on the Arctic tundra ecosystem or by Bormann and Likens on the mineral balance of small catchment areas, apply the systems analysis approach to data obtained and formulate hypotheses about ecosystem relationships. Several of the chapters represent a very real synthesis of ecology with traditional management disciplines like forestry, range management and fish and game management.

The treatment is uneven; there is some overlap, and in some cases there is not sufficient information to allow the application of these principles and methods to one's own problems. It is part symposium, part textbook. But if we are to realize the real practical value of ecology, all ecologists should read this book, in spite of its cost and although they may not find all the answers they are looking for.

P. J. NEWBOULD

INSIDE THE EARTH

Phase Transformations and the Earth's Interior

Edited by A. E. Ringwood and D. H. Green. (Proceedings of a Symposium held in Canberra, January 6-10, 1969, by the International Upper Mantle Committee and the Australian Academy of Sciences.) (*Physics of the Earth and Planetary Interiors*, Vol. 3.) Pp. xi + 518. (North-Holland: Amsterdam, 1970.) 280s.

This volume will be a delight to the Earth scientist interested in the latest state-of-the-art review of our knowledge about the upper and the lower mantles of the Earth. It suffers from the usual problems of conference proceedings, such as delayed publication (one year in this case), juxtaposition of review papers as well as original contributions in highly specialized fields, and sheer volume (518 journal pages with two columns per page) and the commensurate, high price.

There are five sections which describe the physics of the mantle; phase transformations in the deep mantle; magmas, xenoliths and petrology; petrology of the upper mantle and lower crust and tectonophysics. The first and the last sections have the shortest content, seven and six papers, respectively. The other three sections have an average of fifteen papers each. Of these some review papers stand out magnificently for their authors' ability to see "the wood" in spite of "the trees". Among these are Ringwood's 47-page-long article on "Phase Transformations and the Constitution of the Mantle" and Anderson and Sammis's joint paper on "The Composition of the Lower Mantle". No practising or budding Earth scientist can do without these reviews in geophysics. There are also a number of valuable papers summarizing the latest achievements of a particular mathematical experimental technique; among these are Press's paper on the application of the Monte Carlo technique to Earth model building by computers; Kawai *et al.*'s paper on the laboratory techniques to produce static pressures in excess of 300 Kb at 1,200° C, and the separate papers on the petrology of nodules by Kuno and Aoki and by McGregor and Carter. In spite of a strong geochemical flavour, there are enough papers which combine geochemistry, seismology and even theoretical lattice dynamics (O. L. Anderson and Liebermann) to provide a unified picture. Personally, however, I would have liked to see more papers dealing with the solid state aspects of the mantle materials; thermal, electrical and optical properties.

Another interesting inclusion appropriate for such a volume would have been the discussions of papers. As it is, one can only guess at the scintillating comments and ripostes that must have accompanied some of the controversial papers.

It is curious to note that apart from the two valuable papers by O'Hara and Fyfe, there were no other contributions from Great Britain. Surely there are more research groups in Britain that claim to work on the problems of the upper and lower mantle?

SUBIR BANERJEE

ALPINE GEOLOGY

Alpes (Savoie et Dauphiné)

By J. Debelmas *et al.* (Guides Géologiques Régionaux.) Pp. 216. (Masson: Paris, 1970.) 30 francs.

This is the second volume to be published in a planned series of guides to the regional geology of France. Professor Debelmas and his co-authors (H. Arnaud, C. Caron, M. Gidon, Cl. Kerekhove, M. Lemoine and P. Vialon) approach the problem of displaying the geology of the Western Alps by means of itineraries which follow a series of six cross-sections or partial cross-sections roughly normal to the regional strike. The most north-

erly of these covers the Pre-Alps east of Geneva while the most southerly crosses the Ultra-dauphinoise and Briançonnais Zones between the Pelvoux and Mercantour external Hercynian massifs. The sub-alpine zones are treated less fully than other units because of the availability of good modern geological maps and the generally abundant exposure. All routes start from relatively large, well known towns and as far as possible make use of the extensive exposures on the sides of the deep-cut Alpine valleys to demonstrate the regional structure. It is a great pity that the authors have, in the interests of brevity, made this largely the guide for the motorized geologist who needs depart no more than a hundred yards or so from his car to reach most exposures. There are, however, a few concessions to those who prefer to leave the roads.

The guide is beautifully produced with 119 line drawings of panoramas, cross-sections, geological maps and stratigraphic columns. Even the non-French reading geological visitor to the region would find this little volume invaluable for these alone. He who reads French will also be equipped with a modern and authoritative, albeit highly condensed, exposition of the geology, not to mention information on where he may safely park his car. In addition to explaining the geology, a guide must ensure that the traveller follows the intended route and finds the exposures described, without undue difficulty. The directions in this guide seem to be completely adequate, but, not having attempted to follow them in the field, this is merely a charitable guess.

It is worth pointing out that an excellent account in English of the geology of the area covered by this guide is given by Ramsay¹, and other itineraries in the region by Ager *et al.*². Other recent accounts cover the country immediately to the west³ and to the south⁴. An innovation borrowed from the *Guide Michelin* is the assignment of stars, in varying number, to exposures of particular distinction. Undoubtedly with that volume and this, the visiting geologist could plan for himself a memorable tour in Savoie and Dauphiné.

E. R. OXBURGH

¹ Ramsay, J. G., *Proc. Geol. Ass.*, **74**, 357 (1963).

² Ager, D. V., Evamy, B. D., and Ramsay, J. G., *Proc. Geol. Ass.*, **74**, 483 (1963).

³ Middlemiss, F. A., and Moulade, M., *Proc. Geol. Ass.*, **81**, 303 (1970).

⁴ Middlemiss, F. A., Gouvernet, C., Guieu, G., Farine-Taxy, S., Humbert, S., Philip, J., Templer, C., Blanc, J. J., Caron, J. P. H., and Guérard, S., *Proc. Geol. Ass.*, **81**, 363 (1970).

MORE DINOSAUR BOOKS

✓ The Dinosaurs

By W. E. Swinton. Pp. 331 + 8 plates. (Allen and Unwin: London, May 1970.) 75s.

Hunting for Dinosaurs

By Zofia Kielan-Jaworowska. Translated from the Polish. Pp. 177. (MIT: Cambridge, Massachusetts and London, April 1970.) 75s.

MORE than any other extinct animals, the dinosaurs attract the interest of the general public. There is therefore an unusually large market for non-technical books about them, in addition to the scientific market for more scholarly treatises. The requirements of these two types of reader are so different that it is very difficult for a single book to be adequate for both. Dr Swinton seems to have attempted such a compromise, and his book falls squarely between the two stools.

The Dinosaurs includes chapters on the discovery, environment, origin, anatomy, physiology, classification, distribution, diseases and extinction of the dinosaurs, together with four chapters in which the better known members of each group are discussed in detail. The detailed explanations of the osteological nomenclature and the general tone of the book suggest that the author hoped

to attract the more amateur reader. But such a reader will find that many other terms, such as metanephric kidney and Cotylosauria, are used without explanation. He may well also be deterred by the degree of detail given in many sections (for example, the museum numbers of various specimens). These features of the book suggest that it was written with a more professional reader in mind, but even he will find it poorly suited to his needs. Though the student will find much interesting information in the text, he is likely to find the poverty of illustration a great difficulty. The book contains sixty-five line drawings; most are of indifferent quality, some would be incomprehensible to the amateur, and there is not a single drawing of a complete dinosaur skeleton. Even the eight plates of the unmatched restorations of dinosaurs by Neave Parker cannot redeem this deficiency, which makes the book less attractive to the amateur and the student alike. It is, however, up to date, and the professional palaeontologist may find it useful as a more detailed text to be used together with better illustrated books such as those of Colbert, but it cannot be said to be adequate without such support.

Of all the areas in which dinosaurs have been found, none can rival the inhospitability of the Gobi Desert, where expeditions from the American Museum of Natural History found both dinosaurs and a few early mammals in 1922-30. Below the evocatively named Flaming Cliffs, they found the nests of dinosaur eggs which, reproduced in plaster or in photographs, can be seen in many museums. More recently, several joint Polish-Mongolian expeditions have visited the Gobi, led by Professor Kielan-Jaworowska of the Polish Academy of Sciences, and her account of these has now been translated into English. She provides the background to the discoveries by describing very briefly the variety of dinosaur which are known, and noting which types are found in the Gobi. She then gives an account of the organization of the expeditions and describes their daily routine of work and the significance of the specimens which were found.

One must admire the fortitude of the members of the expeditions, who spent long hours in the heat of the desert without even the expectation of frequent discoveries to support their morale—for the mammal remains are far from common and are so small that the only way to find them was "to crawl on all fours with your face close to the ground and to inspect each piece of sandstone under a magnifying glass". As on any such expedition, most of the time was taken up by the routine work of search, excavation and plaster-jacketing. That is inevitable, but, unfortunately, it also inevitably makes for rather dull reading. Though Professor Kielan-Jaworowska provides some interesting information on the life of the inhabitants of the Gobi Desert, and though the book contains nearly sixty photographs, it seems unlikely that many readers would find it worth the outlay of 75s.

BARRY COX

ANALYTICAL EXCHANGERS

Ion Exchange in Analytical Chemistry

By William Rieman III and Harold F. Walton. (International Series of Monographs in Analytical Chemistry, Vol. 38.) Pp. xiii + 294. (Pergamon: Oxford and New York, June 1970.) 130s; \$17.50.

Ion exchange separations unquestionably are of immense importance in modern chemistry particularly in the realm of analytical chemistry. Not a few books and specialist monographs within the general encyclopaedia of analytical chemistry have been devoted to this topic and one automatically makes comparison when another new text is produced on the same subject.

This text stands up to the comparison very well indeed. It is comprehensive in its coverage and is extremely

well written. It also contains that element that is so often missing from modern texts—the authors' own comments on experience. It reviews the history of ion-exchange methods, discusses the synthesis of the materials and gives an excellent account of their physical and chemical properties as well as a good review of the plate-equilibrium theory of the exchange mechanism.

I consider that this is the best book that has yet been produced on the subject of ion exchangers in analytical chemistry. It contains a wealth of theoretical background and practical information and gives all manner of useful hints on the preparation and use of columns, detection methods, and the like. It abounds in diagrams, graphs and tabular data and is as up to date as one could hope for in such a rapidly developing area.

T. S. WEST

REACTIONS ON SURFACES

Catalysis by Nonmetals

Rules for Catalysis Selection. By Oleg V. Krylov. Translated by M. F. Delleo, jun., G. Dembinski, J. Happel, and A. H. Weiss. (Physical Chemistry: a Series of Monographs.) Pp. x + 283. (Academic: New York and London, May 1970.) 131s.

THIS volume is devoted to heterogeneous catalysis excluding catalysis by metals, and is concerned most particularly with reactions occurring on oxide surfaces. The first part discusses the various properties of a solid, electronic (for example, conductivity, work function, band gap, doping, electronegativity difference, d-electron configuration, colour), acid-base, and geometric, which may serve as a basis for the characterization and selection of catalysts. The second part examines a number of practical catalytic reactions including elimination, hydrogenation, dehydrogenation, exchange, oxidation, isomerization, condensation, cracking, and polymerization. In each case a very determined effort is made to correlate published activity with the solid properties discussed earlier and to see which of these are the most significant for defining rules for the selection of catalysts.

The general approach reflects much recent Russian thinking on the empirical principles of catalysis, but the experimental material covered is not restricted to Russian work. The translation seems to have been a little mechanical and provides a quaint taste of the original; but as a result it needs to be read with some previous understanding of the subject matter. The book should be of considerable interest to all workers in heterogeneous catalysis.

C. S. G. PHILLIPS

USES FOR CARBON

Active Carbon

Manufacture, Properties and Applications. By M. Smisek and S. Cerny. (Topics in Inorganic and General Chemistry, Monograph No. 12.) Pp. xii + 479. (Elsevier: Amsterdam, London and New York, 1970.) 225s.

CURRENT publications, devoted entirely to carbon and graphite, make no serious reference to the manufacture and applications of active carbon. This book attempts to make good the omission by discussing the manufacture, properties, structure and applications of active carbons, methods of quality control together with the theory and dynamics of adsorption.

The appearance of the book is timely: carbons generally are emerging from the dark ages of empiricism to play an enlightened part in environmental control. Chemists, chemical and civil engineers will find here collected material of immediate practical interest and of reference

value. It is hoped that the book will stimulate an informed demand for quality control of specific active carbons. Some thirty applications are discussed including solvent recovery, deodorization of air and water, and sugar purification. Industrial manufacturers are developing hard active carbons capable of extensive re-cycling with low abrasion characteristics. The production of specific active carbons requires, at least, control of pore-size and surface functional groups with reliable, realistic means for characterization. The Czech authors have seriously attempted to present theories of adsorption from vapour and liquid states and of pore characterization in terms of carbonaceous systems. The Dubinin theory of adsorption is clearly explained and it is gratifying to read (p. 116), what I have suspected for some time, that this theory is based on an inspired guess. But I must mildly caution readers of the more theoretical sections. These are translations from an earlier book (1964) upgraded by more recent references, but their contents are often not integrated into the text. Hence, a few outdated ideas of a decade ago are perpetuated: comparisons between active carbons and graphite are not realistic (p. 47); the graphite crystallite is only a crystallographic myth (p. 50); the basic concepts of Franklin are now justifiably disputed (p. 55); we have no unequivocal evidence for stating that selective gasification of distinct structural components creates pores (pp. 12 and 55).

HARRY MARSH

SOVIET NEWS

Soviet Science Review

Scientific Developments in the USSR. Vol. 1, No. 1. Bimonthly. Pp. 56. (Iliffe (Science and Technology): Guildford, July 1970.) Annual subscription 300s; \$37.50.

It is difficult to judge a new journal from its first issue, particularly when the subject covered is as wide-ranging as the whole field of Soviet science. A general impression is all that can be gained, and this impression, in the case of *Soviet Science Review*, is a somewhat disappointing one.

The subjects covered range from space medicine to ichthyology and from hypnosis to pollution, but the general effect is that of "prestige" achievements written up for publicity purposes, rather than a serious appraisal of current research. There are some rather extravagant claims and certain inconsistencies, as in the paper on vitreous semiconductors, where the text reads "The experimental research situation is well symbolized in Fig. 1. Point A shows that for some time after the discovery of this new class of glasses, our Institute was the only research body that studied them", whereas in fact (and according to the caption) Fig. 1 represents the possible alloys of metal oxides, sulphides, selenides and tellurides of vitreous and non-vitreous composition, and point A corresponds to an alloy of Tl_2Se and As_2Se_3 .

The bibliographies to the individual papers might well have been composed in the pre-thaw days when scientific isolation was standard Soviet policy. Apart from two references on water pollution, all the works cited are of Soviet provenance, so that it is almost impossible to relate the papers to parallel research in other countries. Another serious defect of the bibliographies is the retitling of Russian works in English (except in the case of the paper on ichthyology) which might well lead to difficulties in tracing the original Russian papers which are the primary source of this material.

Soviet Science Review is published by Iliffe in cooperation with the Academy of Sciences of the USSR. It has an impressive editorial board of Soviet academicians, but the general tone of the publication speaks rather of the *Novosti* news agency (another associated body) than of the academy.

VERA RICH

Correspondence

Poly-I : C

SIR,—As much as anyone, I look toward the advent of specific therapies for viral diseases. Unfortunately, the data reported under the heading "Good News on Poly-I:C" (*Nature*, **225**, 1103; 1970) do not merit the optimistic tone of the title or the article.

One can use Fisher's exact method to calculate the probabilities that the incidences of cold and influenza were the same with placebo as with poly-I:C. The values are $P > 0.1$ and $P > 0.2$ respectively. We really need more encouragement than that.

Possibly Baron *et al.* mentioned this in their presentations (I do not have ready access to copies). If so, this, in all fairness to the authors, should have been stated in the report.

Yours faithfully,

HENRY B. MURPHREE

Rutgers: The State University,
New Brunswick,
New Jersey 08903, USA.

Scientific Bibliography

SIR,—Naranan¹ claims to have provided a mathematical model which exposes the mechanism underlying Bradford's law of scatter of articles relevant to a given subject among scientific journals. In particular he claims that "a frequency distribution $J(p)$ of the number of journals with p articles, of the form

$$J(p) \propto p^{-\gamma} \quad (1)$$

with $\gamma \approx 2$, reproduces the salient features of Bradford's law". Naranan's model certainly reproduces some salient features of the growth of scientific literature on the reasonable assumptions that numbers of journals and of papers in journals are both growing exponentially. But the frequency distribution (1) is only an asymptotic approximation to Bradford's law which others have also considered from time to time. A critical review of recent bibliographic work on the distribution (1), which has arisen independently in several taxonomic fields and which bears several names, has recently been published by Fairthorne². No one has yet succeeded in deriving Bradford's law precisely, in a form which generates the geometric series which Bradford explicitly specified³, from any frequency distribution of the form $p^{-\gamma}$.

However, Naranan's analysis needs only slight modification to provide a model of another empirical law of scientific bibliography first enunciated by Lotka⁴ and based on analysis of entries in a 10-year cumulative index of *Chemical Abstracts*. Lotka found that $A(p)$, the number of authors who contribute precisely p papers, is proportional to $p^{-\gamma}$ with $\gamma \approx 2$. This distribution, it can be seen, is identical with (1) above. If for Naranan's assumption that the number of journals is growing exponentially we substitute the assumption that the number of contributors is growing exponentially (which is equally valid), then Naranan's analysis (with suitable verbal amendments) provides a plausible model of Lotka's law.

Lotka's law, like Bradford's law, is also remarkably stable though the conditions under which it can be expected to apply are as yet less well understood. This "inverse square law of scientific authorship" has hitherto been regarded as an inexplicable and useless statistical oddity. Naranan's model of it is therefore welcome. And, together with other measures of scientific produc-

tivity, Lotka's law has recently been applied by Dobrov and Korennoi⁵ in determining the optimum size of research institutes in the USSR.

Yours faithfully,

B. C. BROOKES

School of Library,
Archive and Information Studies,
University College, London WC1.

¹ Naranan, S., *Nature*, **227**, 631 (1970).

² Fairthorne, R. A., *J. Doc.*, **25**, 283 (1969).

³ Bradford, S. C., *Documentation* (Crosby Lockwood, London, 1946).

⁴ Lotka, A. J., *J. Washington Acad. Sci.*, **16**, 317 (1926).

⁵ Dobrov, G. M., and Korennoi, A. A. in *On Theoretical Problems of Informatics* (F.I.D. 435, Moscow, 1969).

Fitting Boulders

SIR,—Since the publication of my note¹ on fitting boulders in New Zealand, Hills's descriptions² together with correspondence I have received make it clear that the phenomenon is very widespread. Examples from the north Yorkshire coast had been noted by C. Simms, curator of natural history at the Yorkshire Museum, and the following extract from a poem he wrote in 1966 well describes them

"Notice how these sea-shored boulders pack,
not all-sprawled as spate rivers leave them,
but crazy-built, seamed by crablined crack.
With age they wear closer to one another."

Hills² disagrees with my suggestion that salt or ice crystallization is a principal agent involved, and he ascribes fitting boulders to wave action. In my note, I was careful not to rule out wave action as a contributory process: nevertheless, I believe the evidence argues against it being the prime force involved. Hills pointed to examples of fitting boulders at mean sea level where they never dry out, but he is mistaken in believing this necessarily precludes salt crystallization. In the presence of a hot dry wind the rate of evaporation could be such that continual wetting merely leads to replenishment of salt solution, salt crystallization being increased rather than retarded.

Hill's statement that there is a relationship between wave force and the maximum size of fitting boulders needs proper exemplification before it can be accepted. As he indicates, the situation is complex, the maximum size depending not only on wave force but also on height above sea-level. I would add to this the following complex factors: the size of the original boulders, since clearly the maximum size of fitting boulders cannot be greater than that of the source material; whether or not the piles of boulders are on firm bedrock or shifting fine material; the fabric of the boulders; the resistance of the various lithologies to weathering processes in general. The maximum size of fitting boulders at all the localities at which I have observed them has simply been the maximum size of the boulders present at those localities.

The ideal location to test the relative importance of salt/ice crystallization and wave action in the production of fitting boulders would be a freshwater lake where ice crystallization is unknown or rare and salinity always low.

Yours faithfully,

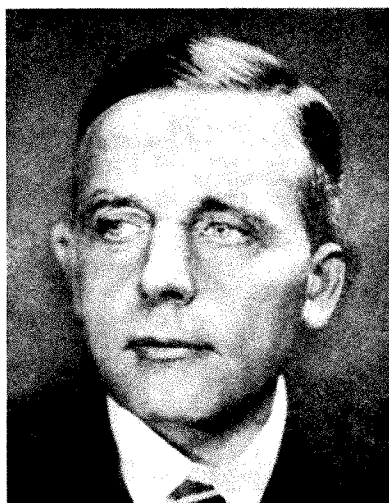
DAVID SHELLEY

Department of Geology,
University of Canterbury,
Christchurch, New Zealand.

¹ Shelley, D., *Nature*, **220**, 1020 (1968).

² Hills, E. S., *Nature*, **226**, 345 (1970).

Obituary



Professor Otto Warburg

OTTO WARBURG died on August 1 in his eighty-seventh year, after a short illness, having remained active in research until almost the end. He is universally acknowledged as the greatest biochemist of this century, even though not every conclusion which he drew from his experiments proved acceptable to the scientific community.

Warburg obtained his PhD in chemistry in 1906 and qualified in medicine in 1911. His main teachers were Emil Fischer, van 't Hoff, Nernst and his father Emil Warburg—the most eminent chemists and physicists of their generation. His genius was recognized early and at the age of thirty, in 1913, he was appointed to a permanent research post in Berlin by the Kaiser Wilhelm Gesellschaft (renamed in 1948 Max-Planck-Gesellschaft). He held this position until his death. The sixty-five year period over which his research extended was interrupted only by war service as a cavalry officer from 1914–1918 and by the period of confusion following the conquest and occupation of Berlin at the end of the Second World War.

His philosophy was, in his own words: “a scientist must have the courage to tackle the great unsolved problems of his time, and solutions have to be forced by carrying out numerous experiments without much critical hesitation”. He applied this to his main research theme, the elucidation of the energy-transforming mechanisms in living organisms. His early work dealt with the study of cell respiration, and he discovered that the respiratory activity of liver is associated with insoluble particles (“grana”) which were identified by Claude thirty years later as mitochondria, structures long known to histologists. In 1923 he discovered the high rate of lactic acid formation by cancer tissue and came to the conclusion that the capacity to obtain energy from a lactic acid fermentation, and to grow at the expense of this fermentation, is a major biochemical characteristic of cancer cells. In 1927 the Nobel Committee considered this discovery of sufficient merit for the award of a Nobel Prize. The committee proposed the division

of the Prize between Warburg and Fibiger, who had discovered the development of stomach cancer in rat infected with the parasitic worm *Spiroptera*. The faculty decided, however, to give Fibiger the undivided prize. The subsequent evaluation of Fibiger's work cast grave doubts on the wisdom of this decision, because *Spiroptera* infection is only one among many irritative stimuli which can cause cancer.

In the later 1920s Warburg, using ingenious and entirely novel techniques, identified an iron porphyrin as the catalyst responsible for the utilization of oxygen in living cells. For this discovery he was awarded the Nobel Prize in 1931. A few years later he identified flavine mononucleotide and flavine-adenine dinucleotide as prosthetic groups of catalysts of biological oxidations and he elucidated their mechanism of action. Connected with this work was the discovery of nicotinamide as the main constituent of the coenzymes of dehydrogenases. In 1944 the Nobel Committee, on account of this work, found Warburg again worthy of a Nobel Prize, but because of Hitler's embargo (he forbade Germans to accept a Prize) the committee did not proceed.

At various times between 1919 and 1970 he was keenly interested in the energetics of photosynthesis, the process on which all life ultimately depends. His main objective was to measure the quantum yield of photosynthesis. He devised conditions under which one molecule of O_2 was released for each quantum of light absorbed by the active chlorophyll. He adhered to the old view that the O_2 was directly derived from CO_2 while the great majority of biochemists have come to the conclusion that the precursor of O_2 is water.

Warburg was a pioneer in biochemical methodology. All his great discoveries rested on the creation of new tools of investigation. The manometric methods for the study of cell metabolism, the modern spectrophotometric methods, numerous microanalytical methods, as well as the procedures used for the isolation of cell constituents and the crystallization of enzymes, sprang from his work. He was also a master of scientific prose; his style was distinguished by an economy of words, by clarity and by strict scientific logic.

Warburg was a striking and fascinating person; he was always friendly, helpful and particularly generous with those genuinely interested in his field of research. Many pieces of research which he initiated and to which he contributed a great deal were published without his name, except in an acknowledgement by the author. He was widely read and well informed in many fields, and his conversation was straightforward, original, penetrating and often had a humorous and highly amusing flavour. The direct look from his bright blue eyes was both engaging and charming; but when it came to scientific argument he was a very forthright fighter for what he regarded as the truth. He crossed swords with many of the leading authorities of his time—Willstätter, Wieland, Keilin among them—and his style in controversy was often scathing and sarcastic. He was motivated by the belief that it was dangerous not to contradict erroneous criticisms.

The many honours bestowed upon Warburg include, apart from the Nobel Prize, Foreign Membership of the Royal Society (1934) and an honorary doctorate of Oxford University (1965).

Announcements

University News

Dr Peter D. Robinson, University of York, has been appointed to the chair of mathematics at the **University of Bradford**.

Professor Julian M. Miller has been appointed chairman of the Department of Chemistry, **Columbia University** in succession to Professor Benjamin P. Dailey.

Professor Dexter S. Goldman, University of Wisconsin, has been appointed professor and director of the Institute for Biology, **Haifa University**.

Dr D. S. Robinson has been appointed to the newly established second chair of biochemistry in the **University of Leeds**.

The **University of Strathclyde** has been awarded a grant of £50,000 by the Nuffield Foundation for the construction of laboratories to house a research group investigating the biochemistry of reproduction.

Appointments

Mr Edward A. J. Rayner, until recently parliamentary press officer to the Conservative Party, has been appointed public affairs executive to the **British Aircraft Corporation**.

Professor C. C. Butler, at present head of the Department of Physics, Imperial College London, has been appointed director of the **Nuffield Foundation** in succession to **Mr Brian Young**.

Mr W. A. Strauss, president and chairman of the Board of the Northern Natural Gas Company, Omaha, and **Dr William H. Danforth**, vice chancellor for medical affairs of Washington University, St Louis, have both been appointed members of the **National Advisory Heart and Lung Council of the US National Heart and Lung Institute**. They will be involved in the evaluation of research and training programmes directed against cardiovascular and lung diseases.

Mr D. J. Flunder of the Dunlop Company Ltd has been appointed chairman of the council of the **National Institute of Industrial Psychology** in succession to **Mr A. J. Nicol**, deputy managing director of Joseph Lucas Ltd.

Lord Kings Norton has been reappointed chairman of the **Council for National Academic Awards**, and will be succeeded in April 1971 by **Mr M. J. S. Clapman**, deputy chairman of Imperial Chemical Industries. The following members of the council have also been appointed: **Mr S. T. Broad**, county education officer, Hertfordshire; **Dr G. Bulmer**, Liverpool Polytechnic; **Sir Derman Christopherson**, University of Durham; **Dr D. Coatesworth**, County Technical College, King's Lynn; **Professor H. C. Edey**, London School of Economics and Political Science; **Mr B. W. Haining**, Food, Drink and Tobacco Industry Training Board; **Dr I. M. Macintosh**, Mackintosh Component Consultants Ltd; **Professor G. D. S. MacLellan**, University of Leicester; **Mr E. Middleton**, City of Birmingham College of Commerce; **Dr Kathleen Ollerenshaw**, Manchester City Council; **Professor M. H. Peston**, Queen Mary College, London; **Lord Pilkington**, Pilkington Brothers Ltd; **Sir Alan Richmond**, Lanchester Polytechnic; **Professor G. D. Rochester**, University of Durham; **Professor D. S. Ross**, University of Strathclyde; **Professor J. H. Smith**, University of Southampton; **Mr K. S. Toft**, City of London Polytechnic; **Reverend G. Tolley**, Sheffield Polytechnic.

Miscellaneous

As from mid-October, the **Science of Science Foundation** will be known as the **Science Policy Foundation**.

Dr Gerhard Herzberg, National Research Council of Canada, has been invited to be the Faraday medallist and lecturer of the **Chemical Society of London** for 1970-71. Dr Herzberg will deliver his lecture on September 30.

Awards amounting to \$1,000 and \$800 were presented by the Division of Psychopharmacology, **American Psychological Association** to two groups of research workers for papers read at the recent convention of the Association. The first prize was won by **Dr K. H. Brodie**, **Dr W. E. Bunney**, **Dr D. L. Murphy** and **Dr F. K. Goodwin** of the US National Institute of Mental Health for their work on drug therapy for the severely depressed patient. **Dr H. B. Cohen**, **J. M. Ferguson**, **S. Henriksen**, **Dr J. M. Stolk**, **Dr V. P. Zarcone**, **Dr J. D. Barchas** and **Dr W. C. Dement** were awarded the second prize for their investigation of the role of 5-hydroxytryptamine in the sleeping pattern of cats.

Mr G. W. Crockford has been awarded the Vernon prize of the **National Institute of Industrial Psychology** for his research on the effects of hot conditions on workers, particularly in the steel industry. The Institute's Miles prize has been won by **Dr Eunice Belbin** for her research into industrial training techniques.

Applications are invited for visiting fellowships to the **Cooperative Institute for Research in Environmental Sciences, University of Colorado**. The Institute is intended to promote research and teaching in solid earth geophysics, oceanography, radio propagation, the physics of the upper and lower atmospheres, and solar terrestrial relationships, and to serve as a centre for multidisciplinary collaboration. Further information can be obtained from the Institute, University of Colorado, Boulder, Colorado 80302, USA.

Research fellowships in forest resources, administered from the **Charles Bullard Fund**, are awarded annually by **Harvard University**. The fellowships are intended to support advanced research by men who show promise of important contributions to any aspect of forestry or forest management—scientific, economic, political, administrative or legal. Candidates will be judged primarily on the quality of their professional accomplishments and academic record. Stipends up to \$15,000 are available depending on the candidate's needs and each fellowship is for one year, not normally renewable. Further information can be obtained from the Committee on the Charles Bullard Fund for Forest Research, Littauer Center 119, Harvard University, Cambridge, Massachusetts 02138, USA.

The **British Nonwovens Manufacturers' Association** (BNMA) was established on September 10 by a group of ten commercial manufacturers involved in bonding processes. The Association aims to provide a basis for cooperation between individual manufacturers and between manufacturers and government, and to consider legislative measures which may affect the industry. **Dr H. A. Thomas**, deputy chairman of Bonded Fibre Fabric Limited, has been elected chairman of the Association; **Mr B. H. Kaufler**, managing director of Bondina Limited will be vice-chairman and **Mr C. F. White**, divisional manager of W. & R. Balston (Industrial) Ltd, honorary treasurer. Further information can be obtained from Dr Thomas, 22 Hanover Square, London W1A 1BS.

British Diary

Monday, September 28

Some Problems and Developments in the Field of Automotive Lubrication (6 p.m.) Mr A. Towle, Institution of Mechanical Engineers, Automobile Division, at 1 Birdcage Walk, London SW1.

Thin Liquid Films and Boundary Layers (three-day discussion meeting) Faraday Society, at the University of Cambridge.

Tuesday, September 29

Autumn Meeting (three-days) Chemical Society, at Imperial College, London SW7.

Trunk Telecommunications by Guided Waves (four-day conference) Institution of Electrical Engineers, in association with the IEEE and the IERE, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Wednesday, September 30

Autumn Conference (three days) Society for Water Treatment and Examination, at Poole, Dorset.

London Discussion Meeting (6.30 p.m.) Society for Analytical Chemistry, Microchemical Methods Group, at Imperial College, London SW7.

The Determination of Trace Amounts of Organic Contaminants in Industrial Organic Materials (6.30 p.m. discussion meeting) Society for Analytical Chemistry, Microchemical Methods Group, at Imperial College, South Side, Princes Gardens, London SW7.

What Constitutes a Truly Integrated Approach to Complete Design (6 p.m. discussion) Institution of Mechanical Engineers, Joint Building Group, at 1 Birdcage Walk, London SW1.

Thursday, October 1

Continuous Fermentation (6 p.m.) Dr M. G. Royston, Society of Chemical Industry, Microbiology Group, jointly with the Institution of Chemical Engineers, the Chemical Society, and the Royal Institute of Chemistry, at the University of Bristol.

Metritation and Paint Packaging in the Future (7 p.m.) Mr R. B. Beazley, Oil and Colour Chemists' Association, at the British Rail School of Transport, London Road, Derby.

Painting of Timber (6.30 p.m.) Mr R. Hill, Oil and Colour Chemists' Association, at the Royal Turks Head Hotel, Grey Street, Newcastle-upon-Tyne.

Recent Developments in Immunochemistry (symposium) Chemical Society, Protein Group, in the Department of Mechanical Engineering, Imperial College, London SW7.

Friday, October 2

Chemical Contraceptives (6.30 p.m.) Dr G. I. M. Swyer, Society of Chemical Industry, Fine Chemicals Group, at 14 Belgrave Square, London SW1.

Recovery and Recrystallization (11 a.m. discussion meeting) Institution of Metallurgists, at the Royal Institute of Naval Architects, 10 Upper Belgrave Street, London SW1.

Reports and Publications

(not included in the monthly Books Supplement)

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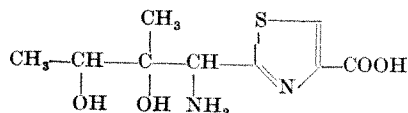
ERRATUM. In the article "Nucleotide Sequences Present within the 16S Ribosomal RNA of *Escherichia coli*" by P. Fellner, C. Ehresmann and J. P. Ebel (*Nature*, **225**, 26; 1970), the figure 69 on line 5, page 28, should read 59.

ERRATUM. In the paper "Genetics of NADP Isocitrate Dehydrogenase in *Paramecium aurelia*" (*Nature*, **225**, 181; 1970), line 16 on page 182 should read "heterozygote pattern (IDH_m 1-2) is consistent with".

ERRATUM. In the article "Stimulation by Cyclic AMP of Intrinsic Protein Kinase Activity in Ox Brain Membrane Preparations", by M. Weller and R. Rodnight (*Nature*, **225**, 187; 1970), the ordinate of Fig. 1 should read: nmoles [³²P]-phosphorylserine/mg protein.

ERRATUM. The author of the article "Effects of Histamine on the Toad Spinal Cord" (*Nature*, **225**, 196; 1970) is A. K. Tebécis.

ERRATUM. The following corrections should be made to the article "The Structure of Thiostrepton" (*Nature*, **225**, 233; 1970): In Table 1, page 234, all minus (−) signs should be replaced by the letter L and plus (+) signs by the letter D; similar corrections should be made in Fig. 2, page 234, and on line 33 of page 235. In Table 1, the chemical formula of thiostreptine is



In ref. 7, the name of the first author is Cross, D. F. W.

ERRATUM. In the article "The Moon at Houston" (*Nature*, **225**, 321; 1970), the second sentence under the sub-heading Chemical Composition on page 326 should read: "Of the ninety-two elements in the periodic table, results are now available for seventy-nine".

ERRATUM. In the article "Activity of Various Fractions of Bradykinin Potentiating Factor against Angiotensin I Converting Enzyme" by S. H. Ferreira, L. J. Green, V. A. Alabaster, Y. S. Bakhle and J. R. Vane (*Nature*, **225**, 379; 1970), the abbreviation pGa on line 12 should read PCA (pyrrolidone carboxylic acid).

ERRATUM. In the article "Growing Pains in British Universities" (*Nature*, **225**, 407; 1970), the second and third sentences in the third paragraph should read: "Fig. 1 shows that between 1959 and 1965, expenditure increased at an average annual rate of about 18 per cent, while student numbers rose by about 7 per cent a year. But after 1965, when Colleges of Advanced Technology received university status, the increase in expenditure seems to have been reduced to about 9 per cent a year, while student numbers continued to rise at an average yearly rate of about 8 per cent".

CORRIGENDUM. At a meeting of the Parkinson's Disease Society on February 11 the impression arose that patients involved in trials of L-dopa would have to stop taking the drug when trials are completed. (*Nature*, **225**, 675; 1970.) Professor J. N. Walton, chairman of the MRC working party on L-dopa, has pointed out that this is not correct, and that patients who benefit from L-dopa have been guaranteed a continuing supply after completion of trials.

ERRATUM. The title of the article by Jay A. Levy and Robert J. Huebner (*Nature*, **225**, 949; 1960) should read "Association of a Murine Leukaemia Virus from a Mouse Lymphoma (2731/L) associated with Reovirus Type 3 Infection".

ERRATUM. In the title of the article by J. Ken McDonald, Benjamin B. Zeitman and Stanley Ellis (*Nature*, **225**, 1048; 1970), the word "appropriate" should read "inappropriate".

ERRATUM. In the review of "A Short History of Botany in the United States" (*Nature*, **225**, 1078; 1970), the second and third sentences of the first paragraph should read: "The eleventh International Botanical Congress was held in Seattle in August 1968. This volume outlines the growth of knowledge . . ."

ERRATUM. In the article "New Finds at the Swartkrans Australopithecine Site" by C. K. Brain (*Nature*, **225**, 1112; 1970), a penultimate sentence should be added to the paragraph beginning "In 1958 . . ." on page 1113: "A concentration of coarse material is visible in the outer cave filling, against the N. wall, just east of the face worked by Broom and Robinson".

ERRATUM. In the article "The Man who Lived with Australopithecines" (*Nature*, **225**, 1188; 1970), it was implied that the stone industry recovered from the Swartkrans cave site was contemporaneous with the Oldowan culture from Bed I at Olduvai, thus making it about 1.75 million years old. The comparison should, however, have been with the much younger culture from Bed II at Olduvai.

ERRATUM. In the article "Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from Pulsars" by Hong-Yee Chiu and Vittorio Canuto (*Nature*, **225**, 1230; 1970), equation 5 should read:

$$\alpha_m = \frac{8\pi^2 c^2}{n_r^2 \omega^2} 2m \int_0^\infty \left[f(p_z') \frac{\partial}{\partial p_z'} \left(\frac{Q_\omega(p_z')}{p_z' + p_z} \right) + f(-p_z') \frac{\partial}{\partial p_z'} \left(\frac{Q_\omega(p_z')}{p_z' - p_z} \right) \right] dp_z' \quad (5)$$

ERRATUM. The book *Matrix Algebra for Business and Economics* was incorrectly priced at 40s (*Nature*, **225**, xxxii; 1970). It costs 140s.

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